Second European Symposium on Lyme Borreliosis

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Lyme borreliosis in the UK

Lyme borreliosis at St George's Hospital, London

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Lyme borreliosis has considerable morbidity both in the USA and mainland Europe. The prevalence of Lyme borreliosis in the United Kingdom is low, though clinical manifestations compatible with the condition are common—for example, facial palsy, aseptic meningitis, and seronegative oligoarthritis. We screened patients presenting to this hospital over a 13 month period with symptoms suggestive of Lyme borreliosis for serological evidence of infection with *Borrelia burgdorferi*. Serum samples were initially tested by enzyme linked immunosorbent assay (ELISA) to detect IgG or IgM antibodies against the flagellin protein (Sigma Diagnostics), and positive samples were immunoblotted to determine whether binding was due to *B burgdorferi* specific antibodies. Serum from 129 patients was tested, 20 of whom had been referred to the Lyme disease clinic. Their main clinical problems were arthritis (in 65), neurological diseases (46), general malaise or pyrexia of unknown origin or both (15), and skin rashes (3). Four patients were under 15 years of age. Cerebrospinal fluid was also examined in nine of the patients with neurological disease.

Raised IgG/IgM antibody levels were detected by ELISA in 13 (10%) subjects: six with arthritis, three with neurological disease, three with general malaise/pyrexia of unknown origin, and one with arthritis with neurological disease. Of these, four (3%) had antibodies to *B burgdorferi* confirmed by immunoblotting and all these subjects had arthritis, one with a concomitant Bell's palsy. Two were children and all (4/20, 20%) were referred directly to the Lyme disease clinic. Three contracted the infection within the United Kingdom and one in Connecticut.

In conclusion, 3% of patients at this London teaching hospital whose serum was tested for antibodies to *B burgdorferi* satisfied criteria for the diagnosis of Lyme borreliosis and this represented 20% of those referred directly to the Lyme disease clinic. All had arthritis and one also had a facial nerve palsy. These data confirm: (*a*) the presence of Lyme borreliosis in southern England; (*b*) the need for a Lyme disease clinic to provide a diagnostic service and treatment; and (*c*) that antibody screening for Lyme borreliosis is not productive.

Evidence of infection with *Borrelia burgdorferi* in urban park workers: a potential new health hazard for inner city inhabitants

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Lyme borreliosis is an uncommon condition in the United Kingdom and cases have previously only been reported from rural areas. Recently, however, ixodes ticks infected with *Borrelia burgdorferi* have been found in Richmond and Bushey parks, London.[1] These parks are close to the centre of the city, have a large population of deer, and are visited by an estimated three million people each year. To determine whether Lyme borreliosis constituted a health hazard to those working in and visiting these parks we questioned 44 of the park workers to assess their exposure to tick bites and evaluate whether they had experienced symptoms compatible with Lyme borreliosis. Their serum was subsequently analysed by enzyme linked immunosorbent assay (ELISA) for antibodies to two different preparations of *Borrelia burgdorferi* (whole cell sonicate and flagellin) and the specificity of these antibodies determined by immunoblotting. Comparison was made with 49 healthy subjects who were either employed in a similar occupation or lived in the same geographic locality. These healthy controls consisted of 27 zoo keepers from a wildlife park outside London, 10 laboratory staff, and 12 bone marrow donors.

Ten of 44 (23%) park workers reported tick bites, and three (7%) described symptoms compatible with Lyme borreliosis. Of these three park workers, two reported rashes characteristic of erythema migrans, followed by symptoms of meningitis and one had an unexplained monarthritis. None of the park workers interviewed had current symptoms compatible with Lyme borreliosis. Significantly raised antibody levels were found in the park workers compared with the healthy controls using ELISA with both purified flagellin (+54%, $p<0{\cdot}001$) and whole cell sonicate (+148%, $p<0{\cdot}05$) as antigen, and 17–24% had antibody levels greater than the mean +2 SD of the controls. Analysis of the immunoblots from park workers and zoo keepers showed that out of a previously reported 14 *B burgdorferi* associated protein bands,[2] more were detected in park workers (mean 1·8, range 0–6) than in the zoo keepers (mean 0·8, range 0–4; $p<0{\cdot}001$), and 32% of the park workers had reactivity with three or more protein bands whereas only one of the zoo keepers showed this level of antigen binding.

These data show for the first time that there is evidence for infection with *B borgdorferi* in workers from these urban parks. This may represent a changing pattern of this zoonosis in the United Kingdom, but whether the increased risk of infection translates itself into significant clinical disease for the visiting population will only be determined by further studies of patients with symptoms compatible with Lyme borreliosis. In the meantime these findings should alert doctors to the possibility that a person who has visited either of these parks and has an unexplained illness may have Lyme borreliosis.

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Pitfalls in the laboratory diagnosis of Lyme borreliosis

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Over a decade has passed since the identification of *Borrelia burgdorferi* as the causative agent of Lyme borreliosis, yet laboratory diagnosis of Lyme borreliosis remains problematic. Conventional bacteriological methods of microscopy and culture have been used with only limited success. Numbers of spirochaetes are too low for diagnosis by direct microscopic examination, and results of culture have been disappointing.

Serodiagnosis

Serology is currently the method of choice for the laboratory diagnosis of Lyme borreliosis. Indirect immunofluorescence and enzyme linked immunosorbent assay (ELISA) have been used for serological screening, but both techniques have their limitations. In a comparative trial we showed that these two methods had similar specificity, but ELISA was more sensitive, less subjective to interpret, and better suited for screening large numbers of samples.[1] Improvements in the specificity of tests have been explored using either purified antigen, such as the 41 kilodalton flagellin protein,[2] or semipurified preparations of *B burgdorferi*.[3] At the same time attempts have been made to increase sensitivity by using antibody capture techniques.[4]

Confirmation of serological reactivity can be achieved by immunoblotting. As the disease progresses antibodies are produced against a range of borrelial proteins, both specific and non-specific. The interpretation of immunoblot patterns is an area of controversy with many different schemes available. Some studies have reported poor specificity of immunoblotting,[5] whereas others maintain that the technique is highly specific.[6] These discrepancies largely result from differing methods used for interpretation. We prefer to use a weighted system for interpretation, with greater value being attached to bands considered to be specific, while other bands are considered less important.[7]

Problems with serodiagnosis arise during

early disease when patients often present with erythema migrans but insignificant levels of antibodies. Of 258 samples submitted to our laboratory with a history of possible erythema migrans, only 16·4% were seropositive. Attempts to improve sensitivity by reducing the cut off level for patients with early disease may not resolve the diagnostic problems encountered with this group. We were unable to show a significantly different distribution in ELISA absorbance readings between patients with erythema migrans and blood donors. Negative results have even been found when serum was tested against the patient's own isolates.[8] The humoral response, unlike the cellular immune response, may be selectively suppressed during early infection.

The IgG response to *B burgdorferi* rises slowly, often not achieving significant levels until the sixth week following infection. Titres of IgG can remain raised years after clinical remission, complicating diagnosis in patients with unrelated clinical presentations.

A major problem encountered with serological tests for Lyme borreliosis is the lack of specificity. Patients with a variety of other spirochaetal diseases and unrelated clinical conditions have been reported to give false positive serological results. Serum samples may cross react by binding to non-specific antigens possessed by *B burgdorferi*, such as the 60 kilodalton common antigen or heat shock related proteins.[9 10]

Considerable diversity exists between strains of *B burgdorferi*, especially among European isolates, resulting in the recent proposition of new genomic species.[11] This may account for differences in seropositivity reported depending upon which strain of borrelia was used as antigen, and highlights the problem of which isolate should be used for diagnostic purposes. Possibly a panel of different isolates should be used for preparing antigens for serodiagnosis?

Other problems encountered with the serodiagnosis of Lyme borreliosis include persistence of the humoral response after resolution of clinical symptoms. Additionally, there are asymptomatic seropositive subjects[12] and those with clinical Lyme borreliosis who fail to mount a significantly raised antibody response.[13] This may result from prompt treatment, which has been reported to abrogate the antibody response.[13] Alternatively, antibody production may be restricted to the site of active disease—for example, in neuroborreliosis the cerebrospinal fluid may be positive but the serum negative.[14] Antibody may remain sequestered in immune complexes and thus not detectable by commonly used assay methods. Cases have been reported with no significant B cell response, but in which specific T cell proliferative responses could be shown.[15]

Antigen detection

Polymerase chain reaction has been used to diagnose Lyme borreliosis with mixed success. Primers used may fail to detect all isolates of *B burgdorferi*, or are not totally specific for *B burgdorferi*. One of the problems with this technique is deciding which sites and how many samples to test before accepting a negative result. Although polymerase chain reaction analysis can provide valuable diagnostic information, its role in the diagnosis of Lyme borreliosis has yet to be fully determined.

Conclusion

Until the problems of sensitivity and specificity can be overcome, results of laboratory findings must be interpreted with caution. The results of diagnostic tests must be considered in conjunction with clinical manifestations before attributing *B burgdorferi* infection as the cause of patients' complaints.

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How to catch Lyme borreliosis: the enzootic cycle for *Borrelia burgdorferi* in Scotland

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Ixodes ricinus, although widespread throughout Scotland, has developed two slightly different life cycles in this region. The east coast maintains the classical two peak life cycle, with flagging producing a majority of nymphs during the spring and early summer months and adult ticks during August and September. The west coast of Scotland, however, has questing ticks throughout the year in addition to the peaks of spring and autumn. The different flora, fauna, and climates of these two coasts may account for these differences.

We analysed *I ricinus* from two sites on the east and west coast for the carriage of *B burgdorferi*, and investigated the prevalence of Lyme borreliosis positive serum samples in a group of workers at high risk based throughout Scotland.

Methods

Ticks were collected from both sites by flagging during May and June. The polymerase chain reaction assay was used to determine, firstly, if *B burgdorferi* was present and, when this was determined, the carriage rate in ticks for the two areas. Ticks were separated into 'nymph' and 'adult' groups before screening to investigate the possible effect of the stage of the life cycle on carriage rate.

Blood samples (5 ml) were collected on four occasions between October and November from each volunteer, and the samples frozen until assayed. Serodiagnosis was carried out using the DAKO second generation Lyme borreliosis kit. Each sample was tested twice, with borderline positives double checked again. All samples proving IgG positive were then tested for IgM antibody.

Tests of significance were performed using standard methods for comparison of the two proportions.[1]

Results

Preliminary data showed that the pooling of ticks for polymerase chain reaction assay underestimated the *B burgdorferi* carriage rate; therefore subsequent analysis was always performed on individual ticks.

Significantly higher carriage rates were found in nymphs than in adult ticks (nymphs 151/500 (30·2%); adults 31/200 (15·5%); $p<0·0001$). Carriage rates were compared for ticks isolated from the west and east of Scotland. Nymphs isolated in the west of Scotland had a significantly higher carriage rate of *B burgdorferi* than those isolated from the east (west 87/250 (34·8%); east 64/250 (25·6%); $p<0·02$). There were no significant differences for adults (west 18/100 (18·0%); east 13/100 (13·0%); NS).

One hundred and fifty three nature conservancy workers from throughout Scotland were tested for IgG seroprevalence against *B burgdorferi*; 29 (19·0%) were found to be positive. A significantly higher seroprevalence was found in workers based in the west of Scotland than in those from the east (west 16/61 (26·2%); east 8/57 (14·0%); $p<0·0001$).

Discussion

The higher carriage rate in nymphs suggests that the tick nymph is a more competent reservoir for *B burgdorferi* than the adult. Our site on the west coast has a large deer

population, and preliminary studies have shown *B burgdorferi* in hind kidney and liver samples. To date no positive samples have been found in stags. The fact that the hinds were culled in August/September at the height of tick activity, and the stags culled in January/February with a much lower incidence of ticks, suggests that the deer do not act as a reservoir for *B burgdorferi*, but are merely an end point in the tick life cycle. Analysis of further samples is required to determine the significance of these results.

The higher carriage rate of *B burgdorferi* in nymphs correlates with data on the seroprevalence from the east and west coasts as our survey of nature conservancy workers showed that they were more likely to be bitten by nymphs than adults.

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Clinical manifestations and treatment

Lyme neuroborreliosis

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Lyme neuroborreliosis is one of the major consequences of untreated erythema migrans and is now one of the most common neuroinfections in Europe.[1 2] Based on strict diagnostic criteria, requiring evidence of cerebrospinal fluid (CSF) inflammation and specific intrathecal antibody production, the incidence in Europe is about 20 cases per million per year.[1]

Neuroborreliosis is not a new disease, as most of the clinical conditions have been described in Europe for many decades. Discovery of the aetiological agent, the tick-borne spirochaete *Borrelia burgdorferi* in 1982, however, led to more specific diagnostic tests, thus enlarging the clinical spectrum of this disease.

Although most patients present with typical clinical features, the diagnostic entity of neuroborreliosis has been distorted in recent years by the inclusion in published reports of many poorly documented conditions. This is mainly owing to the undue popularity of this diagnosis and ignorance of the generally rather low predictive value of a positive serum antibody test. Only strict and generally accepted diagnostic case definitions will avoid such continuing overdiagnosis of this condition.[1-3]

Neuroborreliosis will occur in about 10% of patients with an untreated erythema migrans. It is not yet known whether neuroborreliosis may develop without a preceding erythema migrans, because this, owing to pallor or a hidden location, may easily pass unnoticed. Studies of the evolution of neuroborreliosis show that it is unlikely to develop in patients with longstanding (more than two months) uncomplicated erythema migrans and neither does it develop if an uncomplicated erythema migrans has disappeared spontaneously for more than five to six months.[1] In the latter case antibiotic treatment is no longer recommended. The nervous system is affected most often during early disseminated disease (stage 2); only about 3–5% of patients have chronic (stage 3) neuroborreliosis.[1] In both conditions CSF inflammation with lymphocytic pleocytosis and intrathecal specific antibody synthesis is a major finding.

The main pathological feature in early neuroborreliosis is a polytopic axonal radiculoneuritis with cranial nerve paresis, limb paresis, and radicular pains. The symptoms are dependent on age. Like in herpes zoster neuralgic pains are worst in adults and less pronounced or absent in children, whereas general symptoms of fever and meningism are significantly more common in children. Early neuroborreliosis is in most cases a benign and self limiting condition which even without treatment disappears within two to five months.

Chronic neuroborreliosis is defined by a continuous disease activity and CSF inflammation beyond six months.[1] Most patients have signs of a progressive encephalomyelitis, where signs and symptoms due to a progressive myelopathy dominate, especially an ataxic spastic gait disturbance.[1 4] Chronic neuroborreliosis may also present as chronic meningitis with pronounced general symptoms, weight loss, headache but without meningism or neurological deficits.[1] Patients with chronic neuroborreliosis will always show a significant *B burgdorferi* specific intrathecal antibody production. In early neuroborreliosis a diagnostic intrathecal antibody synthesis begins in the second week after onset and is detectable in 85% of the patients by two to three weeks and always by six to eight weeks after onset of neurological symptoms.[5]

Both stage 2 and 3 neuroborreliosis respond to adequate antibiotic treatment, which should consist of a 10–14 day course of intravenous penicillin G or ceftriaxone. There is no evidence that the therapeutic efficacy of ceftriaxone is greater than that of penicillin. Nor is there evidence that courses of intravenous treatment of longer than 14 days improve the outcome.

It is not yet known whether host or spirochaetal factors determine the course of a *B burgdorferi* infection resulting in either a self limiting erythema migrans in stage 2 neuroborreliosis or in chronic central nervous system (CNS) infection. *B burgdorferi* strains are diverse but neither phenotyping nor genotyping has shown CNS organotropism of certain strains. The probable routes of CNS invasion are by haematogenous spread and by transneural migration from the site of entry. The median time from onset of an erythema migrans to establishment of a CNS infection is three weeks.[1] The direct involvement of the spirochaete is indicated by its presence in CSF and by the prompt effect of antibiotics. However, the mechanism of nerve tissue injury remains unknown. Autoreactive T and B cell responses—for example, to myelin basic protein, occur, but it is not clear whether they are of pathogenetic relevance or just reflect an epiphenomenon. Epidemiological evidence makes it unlikely that *B burgdorferi* persist as a latent CNS infection for years.

Acrodermatitis chronica atrophicans is often (50–60%) associated with a mild, chronic, mainly sensory, distal polyneuropathy.[6] Apart from this distinct condition, there is little evidence of *B burgdorferi* induced peripheral neuropathy without concomitant inflammation of the neuraxis.

In summary, Lyme neuroborreliosis now belongs to the most common forms of bacterial meningitis. The clinical features of both early (stage 2) and chronic (stage 3) neuroborreliosis are usually typical and distinct. Strict diagnostic criteria, including evidence of CSF inflammation, are necessary to avoid overdiagnosis.

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Aspects of Lyme carditis

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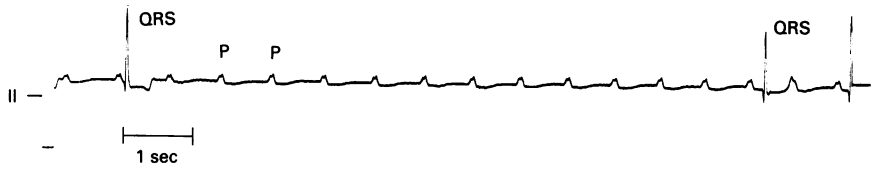
The clinical pattern of Lyme carditis as a manifestation of Lyme borreliosis has been described in many case reports and some review articles. Although Steere and coworkers described most of the aspects of Lyme carditis in 1980,[1] it took some years before this manifestation of Lyme borreliosis was generally known as a possible cause of cardiac problems in everyday clinical practice. Several international conferences on Lyme borreliosis and many local symposia on the subject contributed significantly to the awareness of Lyme carditis as a treatable cause of heart disease. Lyme carditis may occur at any age and in either sex. The incidence of Lyme carditis is estimated as 1·6–8% in patients with Lyme borreliosis.[1-4] Difficulties in confirming Lyme carditis, unfamiliarity with the clinical pattern, and accidental cardiac abnormalities in patients with Lyme borreliosis without causal relation to this disease may lead to under- or overestimation of the incidence of cardiac manifestations in Lyme borreliosis.

Transient and varying degrees of atrioventricular block is the most common feature of Lyme carditis (or might have received most attention). Data of electrophysiological studies show that affection of the cardiac conduction system can be local or rather diffuse.[5 6] Other features are (myo)-pericarditis, heart failure, supraventricular rhythm disturbances,[7] and recently also ventricular rhythm disturbances have been described.[8]

Diagnosis of Lyme carditis can be difficult



Rhythm strip of a 40 year old patient with complete atrioventricular block due to (biopsy proved) Lyme carditis. The instability of the escape focus in this patient is demonstrated by the ventricular standstill of about nine seconds (despite infusion of isoproterenol). A temporary pacemaker was inserted.

because it may be the only manifestation of Lyme borreliosis. A history of a tick bite or erythema migrans is often lacking or not remembered by the patient, and the symptoms of Lyme carditis can be varying, as they depend on the underlying affection of the heart. Alertness of the clinician and familiarity with the clinical pattern play an important part in the diagnosis of Lyme carditis. Laboratory tests, especially immunofluorescent analysis and enzyme linked immunosorbent assay (ELISA) antibody tests of *B burgdorferi*, are still indispensable for the confirmation of Lyme carditis, and in combination with a positive gallium-67 scintigraphy the diagnosis is highly suggestive. When *B burgdorferi* spirochaetes are demonstrated in the endomyocardial biopsy samples (with the silver staining technique or cultivation) of a patient with suspected Lyme carditis, the diagnosis is virtually proved. Electrocardiography, Holter monitoring, echocardiography, and electrophysiological studies disclose the type and extent of the cardiac affection. In patients with conduction disturbances with complaints, hospital admission for continuous monitoring is mandatory because escape foci in Lyme carditis can be very unstable[9] (figure).

The same applies of course in the case of rhythm disturbances with haemodynamic problems and overt heart failure. Antibiotic treatment of Lyme carditis does not seem to be an unconditional requirement for the recovery from Lyme carditis itself. Circumstantial evidence suggests that this treatment cannot be withheld, however, because of the proved beneficial effects in other manifestations of Lyme borreliosis. In some cases a temporary pacemaker is needed, but pacemaker implantation is seldom required, so patience in this respect is necessary. Recently, an overview about aspects of Lyme carditis with respect to 66 cases in Europe was published,[10] in which the therapeutic options in the presence of Lyme carditis are also summarised.[11] The overall prognosis of Lyme carditis seems to be favourable, though delayed recovery has been described and late manifestations (like dilated cardiomyopathy) have been demonstrated by Stanek and coworkers[12] and others.[13]

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Lyme borreliosis in children

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Lyme borreliosis is a common infectious disorder in children. Their daily life and play routines, in particular, make them more likely than adults to be bitten by ticks, and thus more likely to be infected by *Borrelia burgdorferi.* In a serosurvey on 574 healthy children from Lower Saxony/Germany the infection rate, measured by the determination of specific IgG antibody titres, was 2·6% and showed a clear increase beyond the age of 5 years (table).

Age specific prevalence of IgG antibodies against Borrelia burgdorferi in healthy children from Lower Saxony/Germany

Age (years)	*Children tested (n)*	*Prevalence of raised IgG titres (serum) (%)*
< 1	68	2·9
1–2	162	1·2
3–4	112	0·9
5–7	122	4·9
> 7	110	3·6
Total	574	2·9

The whole clinical spectrum of Lyme borreliosis described in adults is also found in children, but age specific differences in the relative incidence of certain manifestations, in the course of the disease as well as in diagnostic peculiarities are evident.[1-7] A prospective, hospital based, multicentre study is presented to describe systematically the epidemiology and clinical spectrum of Lyme borreliosis in childhood with special regard to its neurological manifestations.[8] Lyme borreliosis was diagnosed on the basis of the detection of specific antibodies against *B burgdorferi* using an IgM capture assay developed by Wassmann *et al*[9] and an IgG enzyme linked immunosorbent assay (ELISA) according to Eiffert *et al.*[10]

The study examined 208 children with Lyme borreliosis, of whom 169 had Lyme neuroborreliosis which was diagnosed by the detection of specific antibodies in the cerebrospinal fluid. The yearly incidence of Lyme neuroborreliosis in Lower Saxony was 5·8 cases/100 000 children aged 1 to 13. The manifestation index was 0·16, or one case of Lyme neuroborreliosis for every 620 infected children, compared with the presence of antibodies against *B burgdorferi* for children in the same age group and region. Both the seasonal distribution of Lyme borreliosis, which peaked in summer and autumn, and information about when the tick bites took place point to an incubation period of a few weeks.

The most common manifestation of Lyme neuroborreliosis in childhood was acute peripheral facial palsy, found in 93 (55%) of all cases (fig 1). Lyme borreliosis proved to be the most frequently verifiable cause of acute peripheral facial palsy in children, causing every second case of this disorder in summer and autumn. Bilateral facial palsy was always found to be caused by Lyme borreliosis; thus it can be considered a specific neurological sign of this infection. The second most common manifestation of Lyme neuroborreliosis in childhood was asceptic meningitis in 46 (27·2%) subjects. Lyme borreliosis was the third most common cause of aseptic meningitis in childhood (11·7%) (fig 2). Meningoradiculoneuritis with peripheral nerve involvement (Bannwarth's syndrome) was diagnosed only in six (3·6%) children, though this is the most common symptom of Lyme neuroborreliosis in adult patients.

The head and neck region proved to be the predominant site of tick bites in children, whereas adults experience most bites on their arms and legs. The difference in the site of the infection, as well as the short duration of the illness in children and the early treatment, may partially explain the profound differences in the clinical spectrum of Lyme neuroborreliosis in children and adults.

Nearly all subjects with a positive history of tick bite or erythema migrans, or both, in the head and neck region showed ipsilateral facial palsy, suggesting a direct invasion via the affected nerve by *B burgdorferi.*

Inflammatory changes of the cerebrospinal fluid along with the presence of specific antibodies are essential for a diagnosis of Lyme neuroborreliosis. The presence of IgM antibodies in the cerebrospinal fluid has proved to be the most reliable diagnostic criterion, and is responsible for the early diagnosis of acute Lyme neuroborreliosis in children. The IgM capture ELISA made possible a simple and reliable detection of intrathecal antibody synthesis which could be

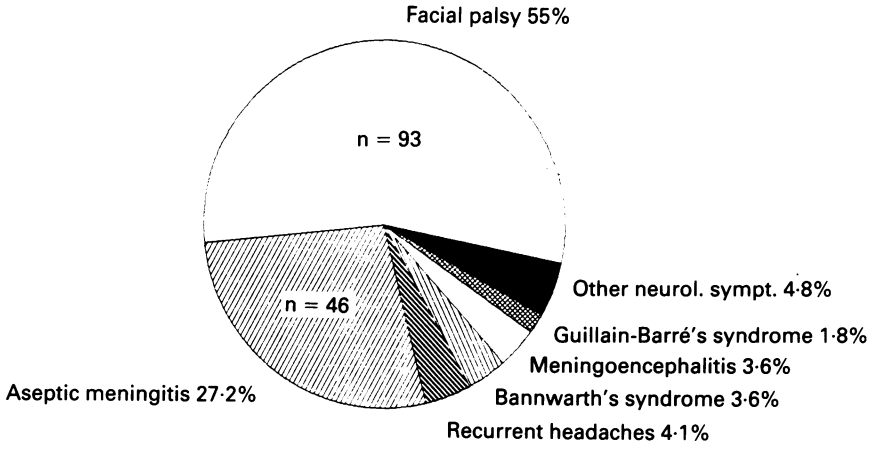

Figure 1 Clinical spectrum of Lyme neuroborreliosis in 169 children.

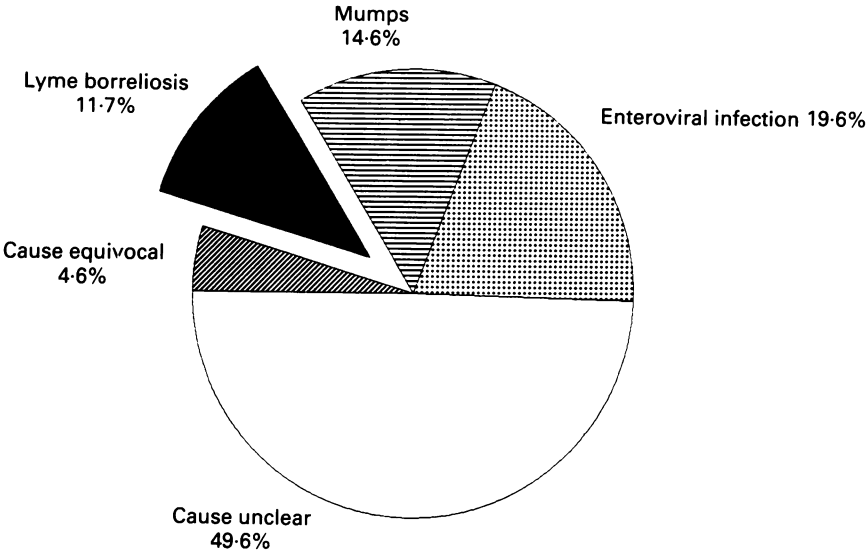

Figure 2 Cause of aseptic meningitis in 240 children. Where the cause is doubtful, cross reactions or double infections are possible.

successfully demonstrated in three quarters of the patients with Lyme neuroborreliosis. Specific IgG testing was negative in most cases because of the relatively short duration of the illness in children, and is therefore of less importance here than in the diagnosis of cases of Lyme neuroborreliosis in adults. High dose intravenous penicillin G proved to be a highly effective treatment for Lyme neuroborreliosis in children.

We investigated 24 children with an erythema migrans and a history of a tick bite at the site of the erythema migrans. Only 11 of 24 children had raised IgM antibody titres, and none of them was positive for IgG antibodies. Five children manifested borrelial lymphocytoma which affected the ears in all cases. IgM antibodies against *B burgdorferi* were found in only two of five children, and none of them had raised IgG antibody titres. Acrodermatitis chronica atrophicans was not seen in our series.

Lyme arthritis was diagnosed in nine children, seven of whom had gonarthritis. Only three of these nine children presented with a history of a tick bite or erythema migrans or both. All children had raised IgM antibody titres, and four had raised IgG antibody titres. In two children with arthritis the diagnosis could additionally be confirmed by an increased IgM antibody titre in the synovial fluid.

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Pathogenesis, diagnosis, and treatment of Lyme arthritis*

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Lyme arthritis was recognised as a new nosological entity in 1975 because of geographic clustering of the disease in children in Lyme, Connecticut, who were thought to have juvenile rheumatoid arthritis.[1] Joint disease in these children was characterised by brief, recurrent attacks of asymmetric swelling and pain in a few large joints, especially the knee. It then became apparent that Lyme arthritis was part of a multisystem illness that usually began with a characteristic annular skin lesion, erythema migrans.[2] This skin lesion had been described previously in Europe, but it had not been associated there with arthritis.

It is now known that joint involvement may be a feature of Lyme borreliosis in both the United States and Europe, but it seems to be a less common manifestation of the illness in Europe. This is probably owing to strain variations in the spirochaete. Subgroup 1 strains of *Borrelia burgdorferi* seem to be particularly arthritogenic. To date, only this strain has been isolated in the United States, whereas all three subgroups of the spirochaete have been found in Europe.[3]

Natural history

During the late 1970s, before the role of antibiotic treatment was known in Lyme disease, we studied prospectively 55 patients with erythema migrans and the initial manifestation of their illness to determine the natural history of the subsequent arthritis.[4] Of the 55 untreated patients, 11 (20%) had no later manifestations of Lyme disease. Ten of the patients (18%) subsequently had intermittent episodes of joint, periarticular, or musculoskeletal pain, but they never developed objective joint abnormalities. After a period of weeks to months 28 patients (51%) had one episode or multiple intermittent attacks of frank arthritis. Most patients developed large knee effusions, but other large joints, the temporomandibular joints, or periarticular sites were sometimes affected. Attacks of joint swelling or periarticular pain were often brief, lasting only weeks. In most cases only one or two joints or periarticular sites were affected at a time. The remaining six patients (11%) developed chronic synovitis later in the illness; of these, two (4%) had erosions, and one (2%) had permanent joint disability.

The number of patients who continued to have attacks of arthritis decreased by about 10–20% each year.[4] Attacks of knee swelling sometimes became longer during the second or third year of illness, however, lasting months rather than weeks. It was during this period that a small percentage of patients developed chronic arthritis, which we have defined as one year or more of continuous joint inflammation. Brief episodes of joint pain sometimes occurred after frank arthritis had disappeared.[5] The total cumulative duration of arthritis was significantly shorter in young children than in adults.

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Pathogenesis

In affected patients *B burgdorferi* probably spreads to joints during the first days or weeks of the illness. Such patients often appear systemically ill and may have vague, migratory musculoskeletal pain in bursae, tendons, muscle, bone, or joints. The specific immune response to the spirochaete is usually minimal.[6 7] Early responses are often restricted to the 41 kilodalton flagellar antigen, the 21 kilodalton outer surface protein C (OspC), or the 58 and 66 kilodalton heat shock proteins of the spirochaete. The specific IgM response, which usually peaks between the third and sixth week of infection, is often associated with polyclonal activation of B cells, including raised total serum IgM levels,[8] and the presence of cryoprecipitates, circulating immune complexes,[9] and occasionally, rheumatoid factor,[10] antinuclear antibodies, or anticardiolipin antibodies.[11] In most instances, these non-specific immune phenomena are not thought to cause symptoms.

Months later the disease seems to localise to one or a few joints, which become markedly inflamed and swollen. By this time patients usually have strong cellular and humoral immune responses to 12 or more spirochaetal polypeptides.[6 7] In the United States the final point in expansion of the antibody response is often the recognition of outer surface proteins A and B (OspA and OspB) near the beginning of prolonged episodes of arthritis, a median of 18 months after disease onset.[12] In a preliminary analysis, expression of IgA antibodies bearing idiotype 16/6 was significantly increased during episodes of arthritis, but not before that time.[13]

As in rheumatoid arthritis, the synovial lesion in Lyme arthritis shows synovial cell hyperplasia, vascular proliferation, and a heavy infiltration of mononuclear cells[14]—a histological picture that is suggestive of a delayed hypersensitivity immune response. In a study by Yssel *et al* all 18 T cell clones studied from patients with Lyme arthritis produced a T helper type 1-like cytokine pattern.[15] This pattern leads to a delayed hypersensitivity response which seems to be important in the control of intracellular pathogens. Preliminary evidence suggests that *B burgdorferi* spirochaetes may survive in fibroblasts and in several other eukaryotic cell types,[16] but the mechanism of their survival in synovial tissue is not yet known. In several instances *B burgdorferi* has been cultured from synovial fluid[17] or seen in synovial tissue,[14] but it has been difficult to demonstrate the spirochaete within the joint by these methods. It is hoped that the polymerase chain reaction may provide a sensitive method to show the presence of the spirochaete within the joint.

B burgdorferi is a potent inducer of interleukin 1 (IL-1),[18] a cytokine with pro-inflammatory effects on synoviocytes and chondrocytes that may lead to destruction of cartilage and bone. Because episodic attacks are characteristic of Lyme arthritis, we studied the clinical course of Lyme arthritis in 83 patients in relation to the synovial fluid concentrations of IL-1β and its natural receptor antagonist (IL-1ra).[19] Patients with high concentrations of IL-1ra and low concentrations of IL-1β had rapid resolution of attacks of arthritis, whereas patients with the reverse pattern of cytokine concentrations had long intervals to recovery. In contrast, concentrations of tumour necrosis factor, a cytokine that shares many biological features with IL-1, showed no correlation with the course of the arthritis. Thus the balance between IL-1β and IL-1ra would appear to be important in recovery from episodes of arthritis.

The fact that only a small percentage of patients develop chronic Lyme arthritis suggests that host factors may determine the severity and duration of the arthritis. To learn whether genetically determined variations in the host immune response might account for such outcomes we determined the immunogenetic profiles of 130 patients with various manifestations of Lyme disease.[20] Of the 80 patients with arthritis, 57% of those with chronic arthritis had the HLA-DR4 specificity and 43% had HLA-DR2; altogether, 89% had HLA-DR2 or HLA-DR4, or both, as compared with 27% of those with only short attacks of arthritis (relative risk 22; p=0·00006). Furthermore, the presence of HLA-DR4 in patients with arthritis was associated with lack of response to antibiotic treatment (p=0·01). In these 80 patients we subsequently showed that only the combination of the HLA-DR4 specificity and antibody reactivity with outer surface proteins A and B (OspA and OspB) of the spirochaete was associated with this prolonged resistance to treatment.[12] In inbred strains of mice an association has also been noted between chronic arthritis and certain H-2 haplotypes, the equivalent of the human HLA-DR allele.[21] These observations suggest that certain class II major histocompatibility genes determine a host immune response that results in chronic arthritis and lack of response to antibiotic treatment.

It is not yet clear whether the situation is the same in Europe. Other subgroups of *B burgdorferi* more commonly cause infection there; antibody responses to the OspA and OspB proteins are unusual, and the occurrence of arthritis, particularly chronic arthritis, seems to be rare.

Diagnosis

The diagnosis of Lyme disease is based upon recognition of a characteristic clinical picture, a history of exposure in an endemic area, and after the first weeks of infection a raised antibody response to *B burgdorferi*. Patients with Lyme arthritis usually have recurrent, brief attacks of oligoarticular arthritis in large joints accompanied by markedly raised antibody responses to *B burgdorferi*; western blots frequently show reactivity with 12 or more spirochaetal polypeptides.[7] In Europe, however, where infection may occur with any one of the three subgroups of *B burgdorferi*, the antibody response may be more restricted. On both continents serological testing for Lyme disease is not standardised and different laboratories may get different results. In addition, patients with past infection often remain seropositive for years; and some patients, particularly in Europe, may have asymptomatic infection. If these patients develop another illness, especially if it is another type of arthritis, the positive serological test for Lyme disease may cause diagnostic confusion.

In adults Lyme arthritis is most like Reiter's syndrome or reactive arthritis, which are types of arthritis associated with HLA-B27. Unlike Reiter's syndrome, we have not seen chronic enthesopathy, sacroiliitis, or urethritis in Lyme arthritis. In children Lyme arthritis is most similar to pauciarticular juvenile rheumatoid arthritis. Lyme arthritis can usually be distinguished from these entities by the brevity of initial attacks and by the markedly increased antibody response to *B burgdorferi*.

A small percentage of patients develop fibromyalgia or chronic fatigue soon after Lyme disease, suggesting that *B burgdorferi* is one of the infectious agents that may trigger these disabling syndromes.[22] Even when triggered by Lyme disease, these syndromes, in our experience, do not respond to antibiotic treatment. Chronic fatigue or fibromyalgia of unknown cause is all too often misdiagnosed as Lyme disease and treated inappropriately with prolonged courses of antibiotics.[23]

Treatment

Lyme arthritis can usually be treated successfully with standard oral or intravenous antibiotic regimens, including doxycycline 100 mg twice a day for 30 days; amoxycillin and probenecid 500 mg of each four times a day for 30 days; or intravenous ceftriaxone 2 g once a day for two weeks.[24] The response may be slow, however, and several months are sometimes required for the complete resolution of arthritis. Regardless of the antibiotic used, the route of administration, or the number of courses given, a small subset of patients with Lyme arthritis does not respond to antibiotic treatment. These patients have an increased frequency of the HLA-DR4 specificity and antibody reactivity to outer surface proteins A and B (OspA and OspB) of the spirochaete.[12] We treat this group of patients with non-steroidal anti-inflammatory agents or intra-articular steroids, or if necessary, with arthroscopic synovectomy.[25]

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Therapeutic options on Lyme borreliosis

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Lyme borreliosis is a vector-borne infectious disease caused by bacteria and thus might be treated with antibiotics. Normally, when treatment of an infection is considered, the pharmacokinetics of the antibacterial drug is judged in relation to the sensitivity of the causative agent. When interpreting the results of sensitivity testing of *Borrelia burgdorferi* it must be remembered that in vitro testing is difficult, and too much weight must not be placed on the results. Thus, for example, macrolides are ineffective in the treatment of Lyme borreliosis despite favourable in vitro sensitivity data. The reverse is true for penicillin, at least according to European experience.

A few animal treatment studies on Lyme borreliosis have been published. The half life of many antibiotics, however, is much shorter in rodents than in humans. Furthermore, treatment for a week or less has been used in rodents, whereas for humans treatment of at least two weeks is usually required. Thus, animal studies can guide us only to a minor extent.

As neither sensitivity testing nor animal studies are reliable we must rely on clinical treatment studies. Several factors must be considered when evaluating a treatment study on Lyme borreliosis:

1 The usual way to verify a bacterial disease in an antibiotic treatment study is to isolate the organism from the patient. This is seldom possible in Lyme borreliosis. Instead, one has to rely on clinical criteria and serology. Serology, especially serum serology, is far from reliable. Furthermore, certain disease manifestations of Lyme borreliosis—for example, arthritis and neuroborreliosis, are difficult to define by clinical criteria.

2 Many symptoms and signs associated with Lyme borreliosis—for example, arthralgias, myalgias, or encephalopathy, are difficult to measure or verify. Such symptoms are sometimes included as end points or inclusion criteria, or both, in studies on Lyme borreliosis.

3 In all stages Lyme borreliosis is a potentially self limiting disease and consequently antibiotics are given to some patients at a time when the patient's immune system has already eliminated the causative agent.

Treatment studies

Erythema migrans In 1980, Steere found that the duration of erythema migrans was significantly shorter in a group treated with antibiotics (four days for penicillin, two days for tetracycline) than in untreated patients (10 days).[1] He later showed that penicillin and tetracycline were superior to erythromycin for treatment of erythema migrans. Åsbrink showed showed in 1986, in a retrospective study of more than 200 patients, that penicillin V was effective for treatment of uncomplicated erythema migrans.[1a] Dattwyler and coworkers compared amoxycillin/probenecid with doxycycline.[2] They found both regimens were equally effective. Hansen and coworkers, in a randomised double blind study, found that treatment with roxitromycin failed in several cases.[3] Penicillin, the comparative drug, was effective and showed no treatment failures. Luft *et al* found that azithromycin was ineffective for treatment of erythema migrans (Luft B J, Luger S W, Rahn D W, *et al*, 5th International conference on Lyme borreliosis, 1992, Arlington, USA).

Neuroborreliosis Sköldenberg *et al* in 1983 demonstrated the effectiveness of intravenous penicillin in patients with chronic meningitis,[3a] later proved to be neuroborreliosis. In 1984 Steere reported on 12 patients with neuroborreliosis successfully treated with high dose intravenous penicillin.[4]

Third generation cephalosporins have been used in several studies. Pfister *et al* compared cefotaxime with penicillin G but found no difference in efficacy between the drugs.[5] In a similar study the same authors found cefotaxime was as effective as ceftriaxone. Dattwyler *et al* found that third generation cephalosporins were superior to penicillin in a study of 23 patients with neurological symptoms or arthritis, or both.[6] Hassler *et al* obtained similar results using cefotaxime.[6a] Differences in inclusion criteria may account for these differing results. Thus in the studies by Pfister *et al* stricter inclusion criteria were used and only patients with meningeal inflammation were included.

Kohlhepp used intravenous doxycycline and found it to be as effective as intravenous penicillin G.[6b] Karlsson *et al* compared oral doxycycline 200 mg daily with penicillin G and found no definite differences. All patients were cured in both treatment groups.

Arthritis Steere was the first to use antibiotics when he showed the effectiveness of penicillin G in comparison with placebo.[7] He found similar results for oral doxycycline, amoxycillin/probenecid, and ceftriaxone.[8] It seems that 60–70% of arthritic patients are cured irrespective of the regimen used. Intra-articular steroids used before antibiotics seem to predispose to treatment failure.

Treatment recommendations

The table lists some recommendations for treatment. The regimen used may depend on treatment traditions in different countries, among other factors. In the USA, particularly, treatment is often prolonged for months or even years, but as far as I know there are no scientific investigations supporting these longer periods of treatment.

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Treatment recommendations for Lyme borreliosis (adults)

Manifestation	*Treatment*
Erythema migrans, uncomplicated	Penicillin V 1·0 g tid*×10 days Amoxycillin 500 mg tid×10 days
Erythema migrans with signs of dissemination, such as multiple erythema or fever	Doxycycline 200 mg daily×10 days
Lymphocytoma, acrodermatitis	As for erythema migrans but prolonged treatment duration to 21 days
Neuroborreliosis	Penicillin G 3·0 g qid*×14 days Cefotaxime 2·0 g bid*×14 days Ceftriaxone 2·0 g daily×14 days Doxycycline (oral) 200 mg daily×14 days
Arthritis	Doxycycline (oral) 200 mg daily×21 days Amoxycillin 500 mg tid+probenecid 500 mg tid×21 days Cefotaxime, ceftriaxone as for neuroboreliosis

*tid=three times a day; qid=four times a day; bid=twice a day.

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Ecology and epidemiology

Ecology of Lyme borreliosis in the United Kingdom

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Comparatively little is known of the ecology of *Borrelia burgdorferi* (the aetiological agent of Lyme borreliosis) in the United Kingdom. Although ecological similarities with continental Europe are apparent, the physical isolation of the UK (as an island), and the different host preferences of *Ixodes ricinus* (the principal vector of *B burgdorferi* in Europe) in the UK, suggest likely differences. Indeed, *I ricinus* is known as the sheep tick in the UK, whereas on the Continent it is called the wood tick.

UK Borrelia burgdorferi

An essential step in understanding the ecology of Lyme borreliosis is defining the properties of the aetiological agent. This is important for selecting suitable diagnostic reagents and in understanding the infection characteristics that influence transmission dynamics. Isolation of UK *B burgdorferi* has proved difficult, and to date only one isolate obtained by Dr S Cutler (Charing Cross Hospital, London) is freely available for study. We succeeded in obtaining only one isolate from 171 tick and vertebrate tissue samples collected in the UK. In contrast, using identical conditions, *B burgdorferi* was isolated from one of seven tick pools from Switzerland, and a single tick pool from Czechoslovakia. The isolation procedures were monitored by polymerase chain reaction (PCR) using a nested set of primers specific for the OspA gene.[1] Eleven of 12 samples were PCR positive after two weeks in culture, but only one sample was positive after eight weeks and motile spirochaetes were not detected by dark field microscopy (Livesley MA, *et al,* unpublished data). The results suggest that the growth requirements of UK *B burgdorferi* differ significantly from those of other *B burgdorferi* strains. One such requirement might be related to the diversity in fatty acid profiles of borrelia (Livesley MA, *et al,* unpublished data and ref 2).

Geographical distribution of infected ticks

To assess the geographical distribution of *B burgdorferi* in the UK a network of tick collectors has been established with the help of Dr B Staines (ITE, Banchory) and Mr R Youngson (Red Deer Commission). Infected ticks have been identified by PCR.[1] The results of this study have been circulated in two Lyme disease newsletters. Although the distribution map (fig 1) is influenced by the activities of the tick collectors, the widespread distribution of infected ticks, extending from Scotland to southern England, is apparent. The distribution of PCR positive ticks broadly corresponds with the recorded distribution of *I ricinus.*[3]

Tick vector species

Although *I ricinus* was by far the most prevalent species collected and diagnosed PCR positive (fig 1), other ixodid species found in the UK were identified as positive (table). The full range of potential vector species must be taken into account in studies of the ecology of Lyme borreliosis. For example, *I hexagonus* has been shown experimentally to be a competent vector of *B burgdorferi,*[4] and in the UK, PCR positive *I hexagonus* have been found in areas (for example, Oxfordshire) where *I ricinus* is not recorded. Thus *I hexagonus* may extend the range of *B burgdorferi* beyond that defined by the geographical distribution of *I ricinus.*

Analysis of the PCR results for *I ricinus*

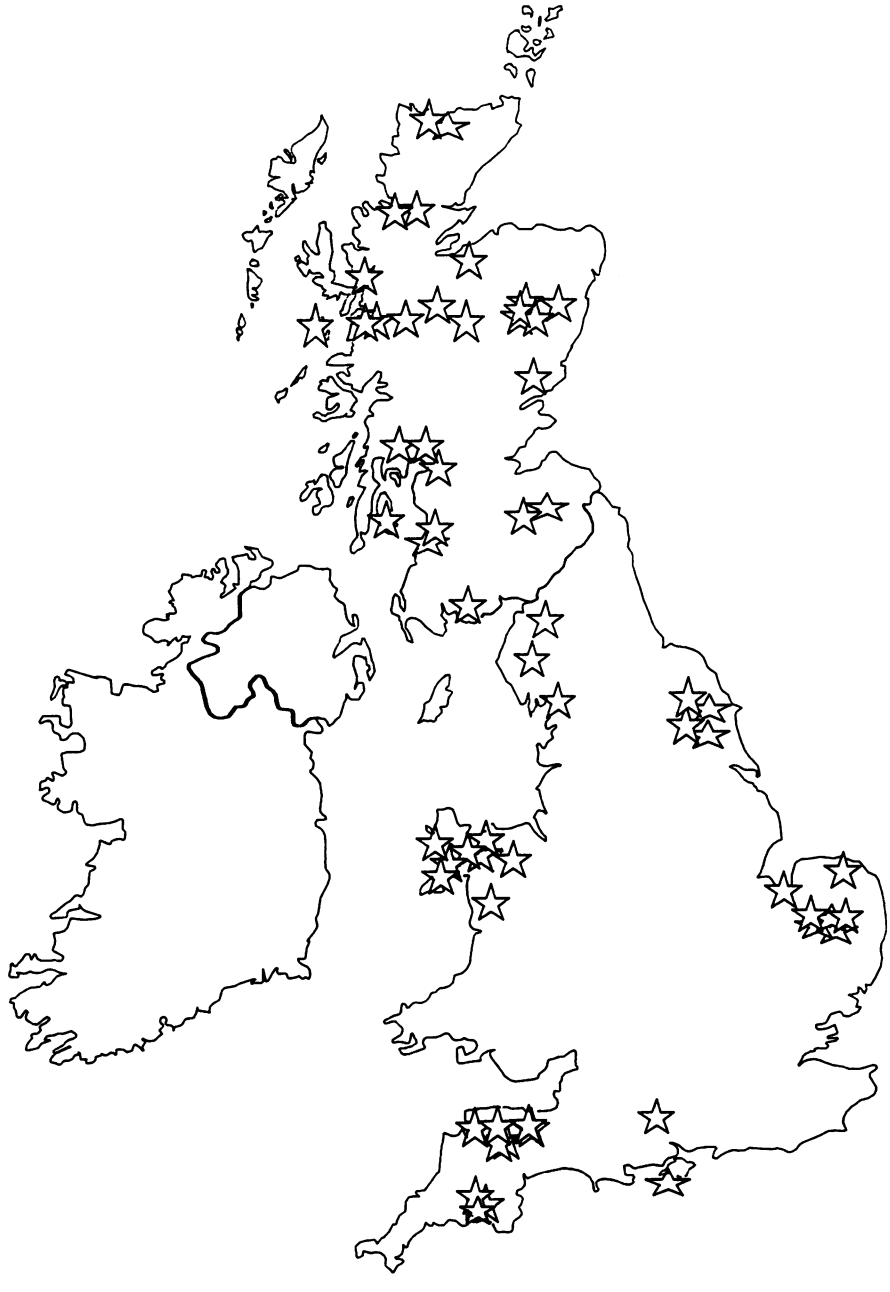

Figure 1 Distribution of Ixodes ricinus ticks collected during 1990 to 1992 and identified as polymerase chain reaction (PCR) positive for Borrelia burgdorferi. Location of PCR positive tick collections are indicated by a star.

Tick species collected in the United Kingdom and diagnosed as polymerase chain reaction positive for B burgdorferi

Tick species	*Location*	*Host associations*
Ixodes ricinus	Most of UK	Numerous vertebrate spp.
I hexagonus	England	Hedgehogs, pets
I uriae	Scotland	Seabirds
Haemaphysalis punctata	Coastal Wales	Sheep

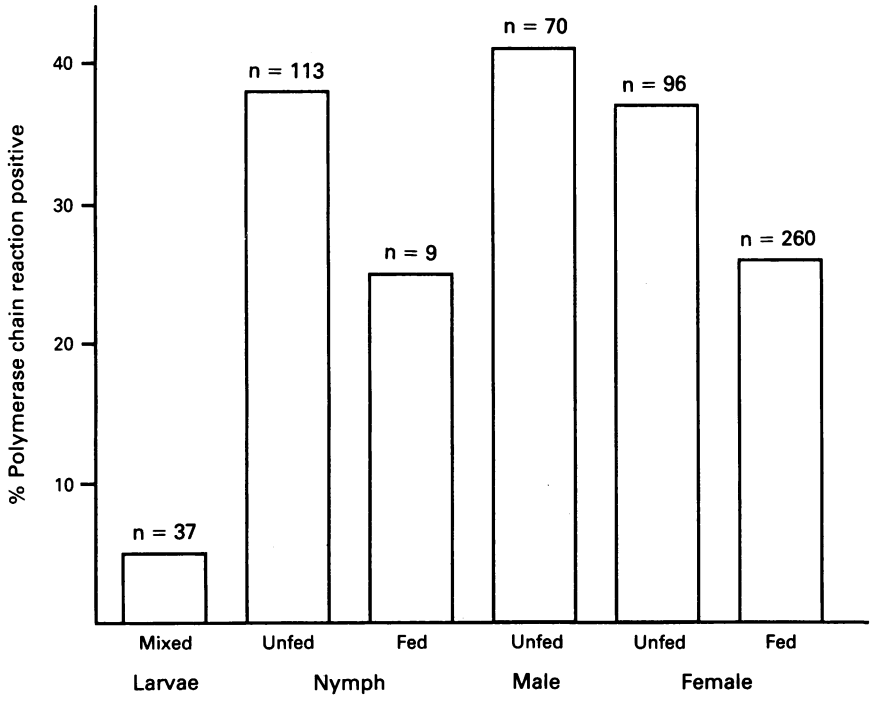

Figure 2 Ixodes ricinus ticks collected in Lyme disease foci identified as polymerase chain reaction positive for Borrelia burgdorferi.

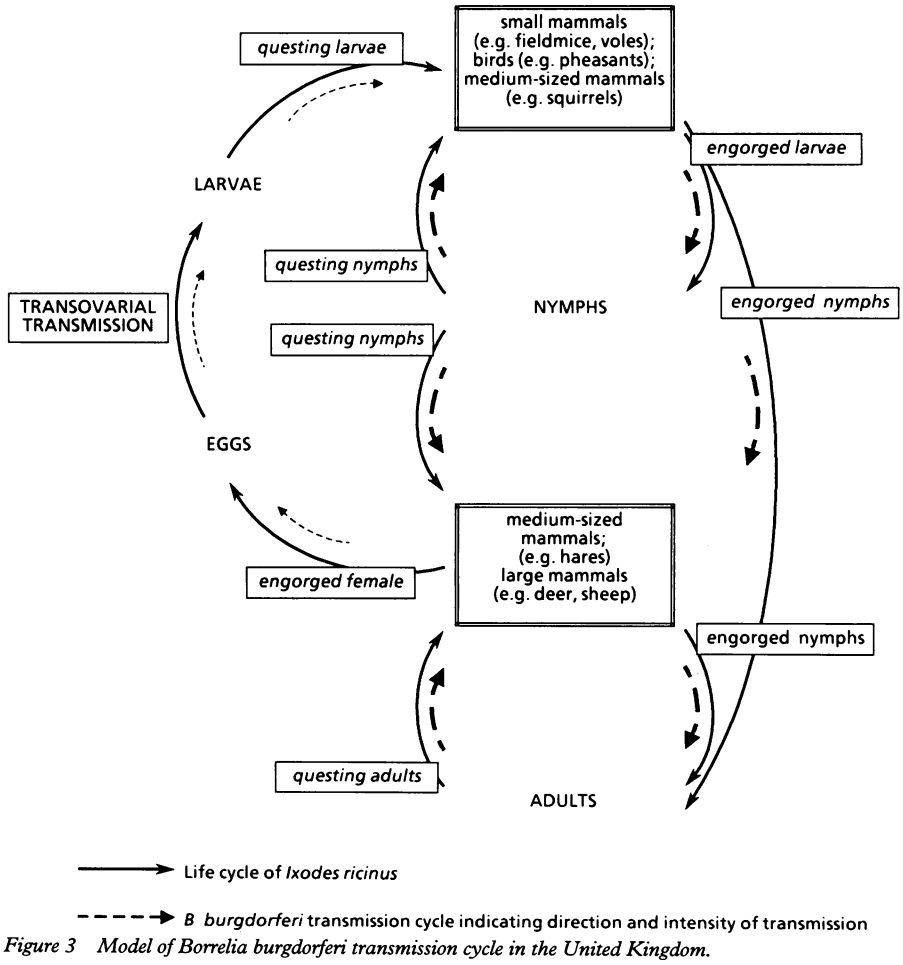

Figure 3 Model of Borrelia burgdorferi transmission cycle in the United Kingdom.

collected in the UK showed a higher percentage of positives among unfed ticks than among engorged ticks of the same stage (fig 2). This is consistent with reports that constituents of the bloodmeal inhibit the PCR reaction.[5] There was little difference in the proportions of positive unfed adults and nymphs, suggesting that most of the infections in the tick population were acquired by feeding larvae and maintained trans-stadially. The detection of two PCR positive pools of questing larvae indicates that transovarial transmission of *B burgdorferi* occurs from the infected female adult to the succeeding tick generation, as described by other workers.[6]

Vertebrate hosts

Most of the feeding *I ricinus* ticks collected in the UK (fig 1) have been from red deer *(Cervus elaphus)* and roe deer *(Capreolus capreolus)*, which we assume reflects a collection bias rather than a specific host preference. In addition, PCR positive ticks have been collected from several other wild and domestic vertebrates—namely, sheep, cattle, horses, dogs, cats, pheasants *(Phasianus colchicus)*, squirrels *(Sciurus carolinensis)*, fieldmice *(Apodemus sylvaticus)*, and bank voles *(Clethrionomys glareolus)*. The susceptibility of these species to infection with UK *B burgdorferi* is as yet largely undetermined, but even species resistant to infection by *B burgdorferi* may have profound effects on the prevalence and transmission dynamics of *B burgdorferi* through their influence on the tick vector population dynamics.

Transmission cycle

The ecology of Lyme borreliosis is defined by the transmission cycle of *B burgdorferi*. Figure 3 shows a model based on our preliminary results. In depth studies in a woodland ecosystem (Thetford Forest) and a sheep upland ecosystem (Cumbria) are being undertaken to test key points in the transmission cycle—for example, the contribution of transovarial transmission to the transmission dynamics. Another important question is the identity of vertebrate host species that ensure transmission from larval to nymphal populations. This requires that larvae and nymphs feed on the same individual hosts. The larva:nymph ratio for fieldmice trapped in Thetford Forest during 1991–92 was 274:1, which is consistent with observations of other workers.[7] In contrast, the ratio on squirrels was 1:3 and on pheasants, 15:1. Thus squirrels and pheasants may be more important than rodents in maintaining *B burgdorferi* in woodland habitats in the UK. The roles of squirrels and pheasants, however, also depend on their tick carrying capacity and their relative abundance in a given Lyme disease focus.

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Geographic diversity of Lyme borreliosis

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Lyme borreliosis is a multisystem disease caused by the tick transmitted spirochaete *Borrelia burgdorferi.* As for other spirochaetal diseases, like syphilis, Lyme borreliosis is characterised by early and late phases, and by protean clinical manifestations that may affect different organ systems, such as the skin, heart, joints, eyes, central and peripheral nervous system.

Symptoms of this infection, primarily affecting the skin and nervous system, were described first in Europe at the beginning of the century. The full clinical picture and worldwide distribution have been recognised only recently, however, after the American reports of arthritis and carditis in patients with erythema migrans, the dermatological hallmark of Lyme borreliosis.

Shortly after, attention was focused on different prevalence figures for the clinical manifestations of Lyme borreliosis, especially for articular disease, which seemed to be more common in American then in European patients. According to data from clinicoepidemiological studies, some of them presented at the Vth international conference on Lyme borreliosis, the approximate prevalence of clinical manifestations of Lyme borreliosis in different geographical areas could be estimated (table).

Prevalence of erythema migrans is similar in the USA and northern Europe, but lower in southern Europe. Acrodermatitis chronica atrophicans and lymphocytoma cutis seem to be almost uniquely reported in Europe, though acrodermatitis chronica atrophicans is quite exclusively present in northern Europe. In Europe the prevalence of arthritis is lower than in the USA, particularly in northern countries. Neurological disease is more common in European patients, while North Americans seem to develop milder forms of nervous system involvement.

Estimated prevalence (%) of clinical manifestations of Lyme borreliosis in different geographical areas

	Skin	*Nervous system*	*Joints*	*Heart*
United States	76	11	46	4
Northern Europe	60	33	7	1
Southern Europe	44	29	21	6
Italy	54	21	30	2

The geographical diversity of Lyme borreliosis not only is concerned with the absolute prevalence of different clinical manifestations but also with the pattern of symptoms affecting any different organ systems.

In European patients with Lyme borreliosis there tend to be fewer clinical manifestations of the disease, even if further clinicoepidemiological studies are needed for confirmation. Various explanations for these differences may be suggested, but the possibility that ascertainment bias might play a part must be considered. Bias may be due both to selection of patients by different specialists (dermatologist, neurologist, rheumatologist) and to low sensitivity and specificity of currently used diagnostic criteria. Unfortunately, laboratory confirmation (mainly based on serological techniques) that a particular disorder is actually caused by *B burgdorferi* infection is at present unsatisfactory. It must also be considered that in endemic areas the presence of a significant background seropositivity might lead to overdiagnosis. On the contrary, subtle or mild symptoms may not correctly be related to Lyme borreliosis, with a resulting underestimated prevalence of the disease.

The first explanation for the geographical diversity of Lyme borreliosis might be that different strains of *B burgdorferi* have a variable pathogenic capacity. The recent delineation of three genospecies of *B burgdorferi* among isolates from Europe, North America, and Japan (*B burgdorferi* sensu stricto, *B garinii,* and group VS461), which tended to correlate with different clinical manifestations, seems to confirm this possibility.

Secondly, the difference may be due, at least for some of the late manifestations, to variations in HLA related host response: in North America chronic arthritis seems to have an immunogenetic basis, being associated with HLA-DR4 or HLA-DR2 specificities.

Finally, European doctors have been treating patients affected with erythema migrans or Garin-Bujadoux-Bannwarth syndrome with antibiotics because previous reports suggested the efficacy of such treatment, and this may well have changed the incidence or the pattern of subsequent clinical manifestations of Lyme borreliosis. Besides, in endemic areas, the use of antibiotics effective against *B burgdorferi* in the treatment of other microbial diseases, might also modify the incidence of clinical patterns of *B burgdorferi* infection.

In conclusion, although geographical diversity of Lyme borreliosis was at first questioned, at present it seems likely to exist. Clinical scientists are now challenged to identify other possible clinical manifestations related to *B burgdorferi* infection.

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Ecology of Lyme borreliosis in Sweden

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The geographical distribution of Lyme borreliosis in the north European countries seems to coincide with the geographical distribution of the principal vector, the common tick *Ixodes ricinus.* We have found that in Sweden this tick species occurs in the southern and south-central parts of the country and along the coast of northern Sweden. This area corresponds with the distributional area of Lyme borreliosis.[1 2] *I ricinus,* and thus also *Borrelia burgdorferi* s.l., are in general not present in the interior of north Sweden, presumably because the climate is too harsh for the vector.

Gustafson and coworkers[3] detected antibody to *B burgdorferi* s.l. in 26% of humans living on the island of Lisö, which is within an area endemic for Lyme borreliosis, located near Stockholm. In contrast, only 2% in a group of healthy blood donors from a non-endemic area were seropositive. In the former group 12% gave a history of previous Lyme borreliosis, mainly erythema chronicum migrans, while less than 1% had had arthritis. This suggests that subclinical infections are common.[3]

B burgdorferi s.l. has in Sweden only been detected in *I ricinus*[4 5] and *I uriae.*[6] Other potential vectors of Lyme borreliosis which we have recorded from Sweden include *I hexagonus, I persulcatus, I trianguliceps,* and *Haemaphysalis punctata.*

Spirochaetal infection in tick larvae in Sweden has not been found, suggesting that transovarial transmission of *B burgdorferi* s.l. is rare. Infection prevalence in nymphs, the principal vector stage, may differ between years and sampling sites.[7] In our studies the prevalence was greater in adults (14–29%) than in nymphs (7–13%) but similar in male and female ticks.[7] The seasonal host-seeking activity of both larval and nymphal *I ricinus* in southern Sweden is generally bimodal with peaks in May–June and August–September, the greatest density being in mixed forest vegetation.[7] The main period of transmission of Lyme borreliosis, however, is during the latter part of the summer and early part of the autumn because at this time both tick activity and human activity—the latter in the form of berry and mushroom picking and hunting—are high in those areas where ticks normally occur.

For Lyme borreliosis to be maintained for a prolonged period of time in an ecosystem two types of hosts should be present: 'reproduction hosts', which provide blood to the female ticks for their reproduction, and 'reservoir hosts', which can infect feeding ticks with *B burgdorferi* s.l. Some mammals, such as hares, function as both reproduction and reservoir hosts. Maintenance of Lyme borreliosis is complex in most mainland ecosystems but relatively simple in island ecosystems where few host species occur.

Our data suggest that roe deer (*Capreolus capreolus*) and moose (*Alces alces*) are important as reproduction hosts for *I ricinus*, but these mammals appear unable to infect this tick with *B burgdorferi* s.l.[8]

In 1987 we isolated *B burgdorferi* s.l. from the yellow necked field mouse (*Apodemus flavicollis*) and the bank vole (*Clethrionomys glareolus*).[9] More recently we have shown that the following mammal species are competent Lyme borreliosis reservoirs: shrews (*Sorex araneus, S minutus*), small rodents (*A flavicollis, A sylvaticus, C glareolus*), and hares (*Lepus europaeus, L timidus*).[10] Small mammals do not usually serve as blood hosts for *I ricinus* females and are therefore not reproduction hosts but only reservoir hosts. In contrast, the hares are usually infested with all stages of *I ricinus*. On isolated islands in the Baltic Sea—for example, Gotska Sandön and Stora Karlsö, the varying hare (*L timidus*) is the only terrestrial mammal present. Our data suggest that dense populations of borrelia infected *I ricinus* are maintained on these islands mainly because of the presence of the hare populations.

A different situation for the maintenance of Lyme borreliosis has been found on the isolated island, Bonden. Large seabird colonies breed on this island located in the northern Baltic Sea. In July 1991 we collected 17 *I uriae* ticks from two juvenile razorbills (*Alca torda*). By phase contrast microscopy spirochaetes were detected in three of the ticks. After using immunofluorescence techniques and polymerase chain reaction DNA amplification followed by sequencing parts of the flagellin and the OspA genes we concluded that the spirochaetes belong to *B burgdorferi* s.l. As there are no mammals on the island nor any *I ricinus* population present it is likely that the spirochaetes at Bonden are cycling between seabirds, principally auks, and *I uriae*. This tick species is associated with colony nesting seabirds in both the northern and southern hemisphere. It infests many species of seabird, including far-ranging species such as the wandering albatross, fulmars, and gannets. This suggests that seabirds may transmit Lyme borreliosis between the northern and southern hemisphere or vice versa and that hitherto unknown foci of Lyme borreliosis may exist.

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Biology and immunopathogenesis

Physical and genetic maps of the *Borrelia burgdorferi* sensu lato chromosomes

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The spirochaete *Borrelia burgdorferi* was discovered in 1982 as the agent of Lyme borreliosis.[1] Since then, three species have been delineated—namely, *B burgdorferi* sensu stricto, *B garinii*, and genospecies VS461.[2] The only species found in the USA is *B burgdorferi* sensu stricto while all three species are found in Europe.

The lack of a system for genetic transfer has hindered conventional analysis of spirochaetes at the genetic level. Analysis of large DNA fragments is possible, however, using pulsed field gel electrophoresis. To determine whether differences exist at the chromosomal level we constructed both physical and genetic maps for each species to enable comparison of their chromosomes. The first results from *B burgdorferi* sensu stricto indicate that the ribosomal genes have a central location within the linear chromosome.[3] Other genes isolated by us or others have been located throughout the chromosome.[4] The physical maps of *B burgdorferi, B garinii*, and genospecies VS461 chromosomes show differences in restriction enzyme patterns, though the genetic maps are quite similar (Ojaimi, Old, Saint Girons, Davidson, unpublished results).

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A mouse model for *Borrelia burgdorferi* infection: approach to the understanding of pathogenesis and protection

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The mouse model for *Borrelia burgdorferi* infection has shown that viable *B burgdorferi* induce chronic arthritis, carditis, myositis, and hepatitis in mice with severe combined immunodeficiency but not, or only to a much lesser extent, in various normal inbred strains of mice. Adoptive transfer experiments showed that mice with severe combined immunodeficiency, reconstituted with either unselected spleen cells, selected B cells, or monoclonal antibodies to OspA and OspB but not those reconstituted with selected T cells or monoclonal antibodies to flagellin, heat shock protein 60 (hsp60), and hsp70, are protected against development of disease upon challenge with *B burgdorferi*. These findings indicate that antibodies to outer surface protein A (OspA) and OspB are important elements in the control of *B burgdorferi* and suggest that a protective polypeptide vaccine from OspA/OspB can be developed.

During infection, spirochaetes disseminate from the site of the tick bite, probably through the bloodstream, and localise in various tissues. It is suggested that during their extravasation *B burgdorferi* organisms activate endothelial cells to express adhesion molecules and cytokines which are required for the attachment of inflammatory cells to vessel walls and for their immigration into the tissue. We have now shown that *B burgdorferi* induces the expression of adhesion molecules such as ELAM-1, GMP-140, ICAM-1, and VCAM-1 in the heart and joints of mice with severe combined immunodeficiency, and that most infiltrating cells are macrophages. Together with the finding that *B burgdorferi* organisms can induce bioreactive molecules such as O_2^- and NO, as well as the cytokines interleukin 1 and tumour necrosis factor α in macrophages in vitro, the data suggest that these processes contribute to extravasation of both macrophages and *B burgdorferi* into the surrounding tissue and to the induction and progression of tissue destruction.

Cellular immune reactions to *Borrelia burgdorferi*: the T cell-macrophage axis

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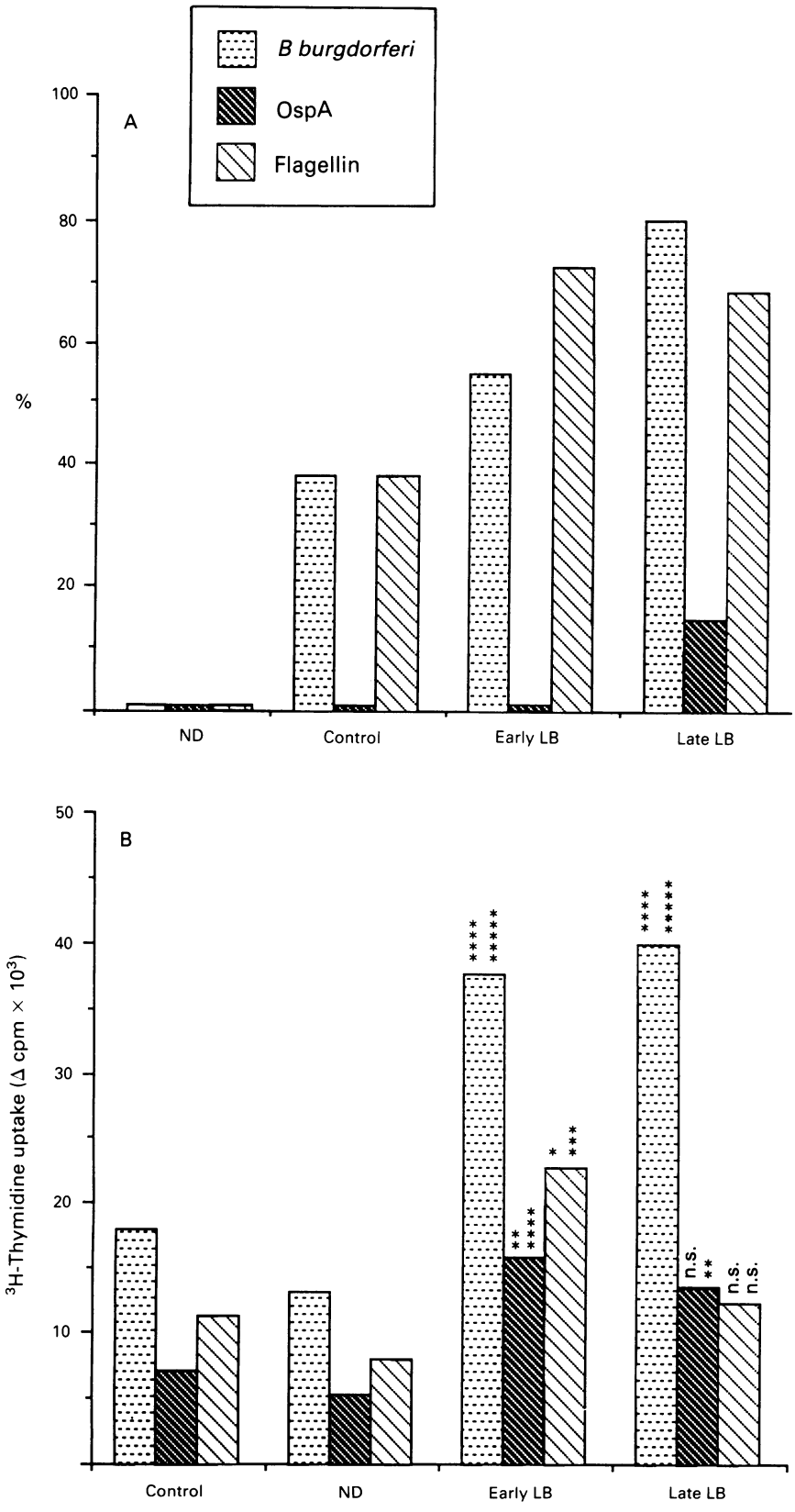

*Figure 1 (A) Humoral immune reactivity to Borrelia burgdorferi in normal donors (ND), subjects without Lyme borreliosis (control), and patients with early or late manifestations of Lyme borreliosis (LB). Reactivity to Borrelia burgdorferi was determined by the immunofluorescence assay and by immunoblotting against OspA and flagellin. The relatively high incidence of positive donors in the control group is apparently influenced by the referral pattern to our outpatient clinic. (B) Parallel cellular immune response against Borrelia burgdorferi, OspA, and flagellin in the same groups as described above. *p<0·05; **p<0·02; ***p<0·008; ****p<0·002; *****p<0·0005.*

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Even though Lyme disease has been clearly identified as an infectious disease, there are still many riddles to be solved, especially with regard to the immune response to this organism. These include the question why only a small proportion of subjects originally exposed to *B burgdorferi* progress to later disease stages, the genetic background, and the apparent dissociation between the cellular and humoral immune reactivity and a tremendous, but nevertheless restricted and apparently non-protective, antibody repertoire.

Most patients with Lyme borreliosis develop an early and strong cellular immune reactivity to *B burgdorferi* that in early stages often precedes an only slowly evolving humoral response.[1] Thus, although in early cases serology may still be negative, in later stages, especially arthritis, nearly all patients are seropositive, with only rare but clinically important exceptions. In a study of the immune response two major groups of proteins of *B burgdorferi*, the outer surface proteins (OspA and OspB) and flagellin, seem to be of particular interest. The spirochaete specific OspA is a 31 kilodalton protein which is expressed in large amounts on the outer membrane of *B burgdorferi*. It is antigenetically variable between different isolates of *B burgdorferi*.[2] Despite its abundance on the bacterial surface, in humans the humoral response to OspA as detected by immunoblotting is quite rare, and, if present, develops only at later stages.[3] In contrast, antibodies to flagellin, the internally localised flagellar protein of *B burgdorferi*, are detectable in nearly all patients with Lyme borreliosis, even in early stages of the disease.[4]

A comparison of the humoral immune responses with the cellular immune reactivity to these two major proteins of *B burgdorferi*, OspA and flagellin, in patients with Lyme borreliosis led to several interesting observations (fig 1).[5] Thus patients with Lyme borreliosis showed a significantly raised T cell response to whole *B burgdorferi* bacteria as compared with patients with other inflammatory joint diseases and with normal controls. This increase was already detectable in very early disease stages and did not correlate with the presence of antibodies against *B burgdorferi*. The *B burgdorferi* specific T cell responses noted included those directed against OspA and flagellin. There was a considerable variation of proliferation values, however, ranging from a lack of T cell proliferation to very high responses. Irrespective of the disease stage, in vitro T cell responses to either whole *B burgdorferi* or to the recombinant proteins did not correlate with the presence of specific antibodies in the patients' serum samples.

This dissociation of the humoral and the cellular immune response may be explained by several mechanisms. Initial experiments using T cell clones in patients with chronic Lyme disease suggest a selective activation of a certain T cell subset that produces a restricted pattern of cytokines which are unable to activate B cells.[6] This selective expansion of responding cells may depend on the HLA profile as suggested by the high association of chronic Lyme arthritis refractory to antibiotic treatment with certain HLA-DR2 and DR4 alleles.[7] In addition to an unusual T and B cell response, there is a peculiar uptake mechanism of *B burgdorferi* by phagocytes, which may explain some of the subsequent specific immune reactions. All types of phagocyte internalise the spirochaetes preferentially through a special uptake mechanism designated 'coiling phagocytosis' (fig 2). In this phagocytic mechanism sheath-like cell protrusions wrap around sections of spirochaetes in multiple turns. These lead to cylindrical coil-shaped complexes, which are subsequently engulfed. The clefts between the pseudopod rotations

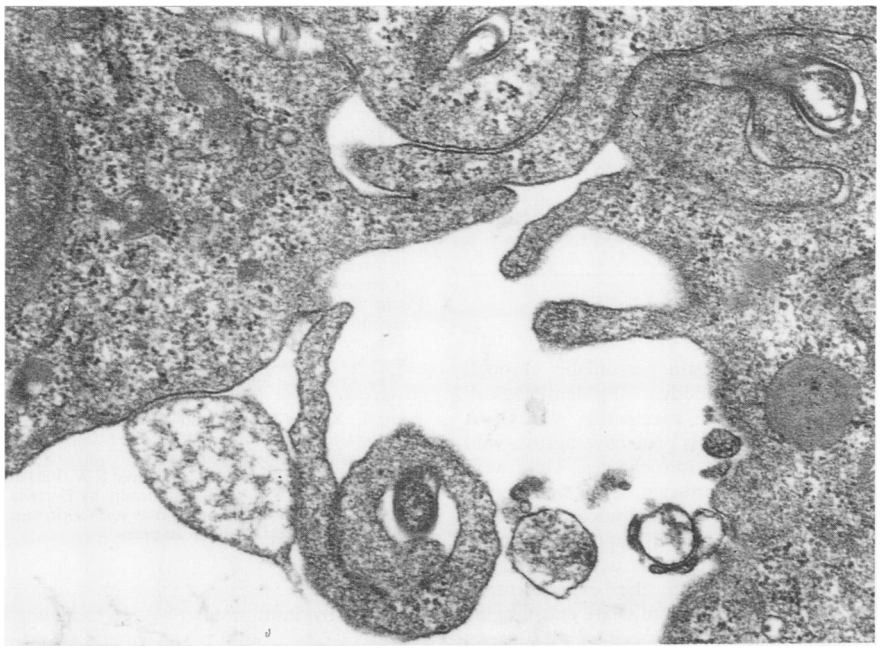

Figure 2 Coiling phagocytosis of Borrelia burgdorferi by a human blood monocyte. The borrelia has been cut in a transverse section and is wrapped by a coil-like protrusion from a monocyte.

are continuous with the extracellular space until the adjacent cell membranes of the coiled pseudopods fuse and dissolve. Spirochaetes internalised by this mechanism disintegrate without visible participation of lysosomes. During conventional phagocytosis, sections of spirochaetes are dragged into the phagocytes and trapped in large sized vacuoles. Again, continuity with the extracellular space is maintained by means of the clefts between the invaginated cell membranes. These clefts subsequently close by membrane fusion. Some spirochaetes already start to disintegrate when these phagosomes are still connected with the cell surface, possibly implying a leakage of lysosomal material into the surrounding environment. Thus the engulfment of the spirochaetes involves extensive membrane events, which are rarely evident during the uptake of other material. Studies using this model will provide new insights in membrane processing during phagocytosis which may be important for the presentation of bacterial antigens in the context of major histocompatibility complex class I antigens.[8]

It is an interesting finding that in the late stages of spirochaetal diseases, such as syphilis and, notably, Lyme disease, few infectious organisms are detectable in the lesional sites. There is often a strong local immune response, however, with a sometimes vigorous synovial inflammatory hypertrophy in Lyme arthritis which is histologically indistinguishable from rheumatoid synovitis.[9] Apparently, just a few bacteria are sufficient to attract vast numbers of cells to the areas affected. It may be either a general immunosuppressive state that does not allow for a complete elimination of the microbe—or, alternatively, the immune system is directed into the wrong direction and uses false or inadequate means for elimination. Thus the study of Lyme disease, especially its arthritic manifestations, has provided important lessons about an inadequacy of the immune response towards elimination of microbial organisms in a chronic infection. These observations may provide important insights into other arthritides which are triggered by contact with infectious agents.

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Contribution of the antibody humoral response analysis to the nosological approach of Lyme borreliosis

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Since 1985[1] variation in the clinical features of Lyme borreliosis has been a controversial subject, particularly between European and North American authors.[2]

An increasing number of studies on borrelia isolates from both humans and ticks have contributed to the rapid improvement of knowledge about the genotypic and phenotypic diversity. A recent description of three genomic species in Lyme borreliosis agents[3] asks whether a given borrelia species might be related to a particular clinical feature.

An analytical technique, the western blot, was chosen to investigate the antibody humoral response of patients with Lyme borreliosis. Serum samples of 67 patients with Lyme borreliosis classified according to their clinical features were analysed using nine strains belonging to the three genomic species as antigens.

The antibody response was shown to be homologous within each genospecies.

For quantitative analysis, serum reactivity was studied against the three type strains: *Borrelia burgdorferi* sensu stricto (strain B31^T), *Borrelia garinii* (strain 20047^T), and group VS461 for the 67 patients. Considering the number and the intensity of the bands 11/23 (48%) of the serum samples from patients with meningoradiculitis showed preferential reactivity against *Borrelia garinii* (strain 20047^T), 8/8 (100%) samples from patients with acrodermatitis chronica atrophicans against the group VS461 (strain VS461), and 11/20 (55%) of the samples from arthritic patients against the *Borrelia burgdorferi* sensu stricto (strain B31^T) (table). The presence of a strong response against OspA and OspB proteins (31 and 34 kilodaltons) of *Borrelia burgdorferi* sensu stricto was found only in this last group of patients. In the meningoradiculitis group the preferential reactivity was partly due to a strong response against the pC protein (22 kilodalton). In the group with acrodermatitis chronica atrophicans all patients showed a strong response against a low molecular protein of the VS461 type strain.

Previous studies have reported the importance of strain choice for investigation of the antibody humoral response by western blot or by enzyme linked immunosorbent assay (ELISA).[4–8] The strains used in these studies could be retrospectively assigned to the three genospecies, and thus their results are in agreement with ours.

Our experience since 1985 shows a remarkably stable repartition of clinical forms. Most of the patients had a sole complication: meningoradiculitis or arthritis or lymphadenosis benigna cutis or acrodermatitis chronica atrophicans.

These data suggested an alternative scheme for the natural history of Lyme borreliosis. Each clinical syndrome is possibly associated with a given genospecies.

A syndromic approach seems to be helpful for understanding the epidemiological differences over the world.

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Western blotting preferential reactivity for each clinical class according to the genospecies of the strain used as antigen (n=67)

Strain (genospecies)	*Erythema migrans alone (n)*	*Meningo-radiculitis (n (%))*	*Arthritis (n (%))*	*LBC* (n)*	*ACA* (n (%))*
Preferential reactivity with:					
B31 (*Borrelia burgdorferi* sensu stricto)	1	2	11 (55)	0	0
20047 (*Borrelia garinii*)	4	11 (48)	2	1	0
VS461 (group VS461)	2	3	2	3	8 (100)
No preferential reactivity	5	7	5	0	0
Total for each class	12	23	20	4	8

*LBC=lymphadenosis benigna cutis; ACA=acrodermatitis chronica atrophicans.

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Protective immunity in Lyme borreliosis

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We are interested in understanding the protective immunity in Lyme borreliosis with a view towards the development of a safe and efficacious vaccine, and determination of useful and specific diagnostic symptoms. C3H mice provide a useful model, for when they are infected with *Borrelia burgdorferi* they exhibit both arthritis and carditis symptoms characteristic of human disease. Moreover, spirochaetes can be cultured from infected animals, which together with disease symptoms provides a useful assay for the assessment of protective immunity. Mice immunised with recombinant outer surface protein (Osp) A and B are protected from challenge with borrelia. This protection is mediated, at least in part, by neutralising antibody, and extends over a broad variety of borrelial strains. One American strain (for example, strain 25015) does not show cross protection when OspA from strain N40 is used as an immunogen, but OspA 25015 protects against infection with *B burgdorferi* 25015. Immunity against *B burgdorferi* also protects against infection via the natural vector, the deer tick *Ixodes dammini*. Interestingly, this experimental vaccine protects by means of two mechanisms: the first eliminates spirochaetes directly within the tick—we believe that this occurs by the uptake of neutralising antibody into the tick followed by destruction of spirochaetes in situ. The second mechanism involves the destruction of spirochaetes within the vertebrate host, again presumably through neutralising antibody. 'Epidemiological' studies suggest that vaccination with OspA N40 or OspB B31 is protective against wild ticks infected with spirochaetes. Ticks were obtained from Nantucket, USA, and placed upon vaccinated or control mice. Vaccinated mice were not infected, and spirochaetes were destroyed within the ticks in precisely the same way with the experimentally infected ticks. These results are encouraging for the prognosis of this vaccine for it suggests that, at least in the American population, spirochaetes are sufficiently homogeneous for protective immunity to be obtained against ticks from at least one geographic location. We are currently examining other locations.

Humans raise antibodies against OspA and OspB, though these antibodies are obtained late in infection and not in all subjects. To test whether such antibodies are protective in principle against infection with borrelia, passive transfer was performed with antibodies from patients, and mice were subsequently infected with borrelia. Mice that had received the human antibodies were protected against infection, provided that the antibodies were derived from patients with high titre and late infection; patients with early Lyme disease seemed not to have protective antibodies.

Diagnosis of Lyme disease is made difficult by the cross reactivity of given antigens with other spirochaetes and by the fact that certain specific antigens only show serological reaction in a minority of patients. We used fragments of flagellar antigen to show that an internal epitope (P41G; 197 to 273) offers a specific diagnostic test with greater specificity and equal or better sensitivity than that offered by the standard whole organism enzyme linked immunosorbent assay (ELISA), or full length flagellar antigen.

We are analysing mechanisms whereby borrelia evades protective immunity. At least one mechanism seems to include mutation of the outer surface proteins to prevent recognition by protective antibodies. Thus *B burgdorferi* strain N40 contains organisms with a truncated OspB which eliminates the C terminal portions of that molecule. Since protective immunity to OspB (and to OspA) is mediated by antibodies directed at the C terminus, such spirochaetes cannot be killed by neutralising antibody and can infect mice vaccinated with full length OspB. It is likely that such mechanisms are used by the spirochaete and we are currently analysing spirochaetes which can be cultured from vaccinated animals to test mechanisms of protection.

Finally, we have identified the genes for several new outer surface proteins which show structural features related to those of OspA, B, C, and D; these proteins are being currently evaluated as diagnostic and vaccine candidates and will be discussed.

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Diagnosis

Serodiagnosis of Lyme disease in the United Kingdom

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The serodiagnosis of Lyme disease is complicated by the often poor immune response in early infection and the relatively high prevalence of cross-reacting antibody in the normal population. The IgM response often does not peak until four to six weeks after infection, but at six weeks most patients are seropositive both for IgM and IgG by enzyme linked immunosorbent assay (ELISA).[1] More than 50% of the normal population were shown to possess detectable levels of antibody reacting with the flagellin component of *Borrelia burgdorferi* by immunoblot.[2] Other infections that can cause cross reaction include *Treponema pallidum, T denticola, T phagedenis, T vincentii, T scoliodontum,*[3] most other *Borreliae* spp, mumps, and infectious mononucleosis. In addition, *B burgdorferi* possesses a number of antigens related to the heat stress/shock class of proteins that are also highly cross reactive.[4] In some serological tests false positives may be obtained as a result of other factors, such as the presence of rheumatoid factor and antinuclear antibodies.

One recent approach to improving the specificity of an ELISA has been the use of purified borrelial antigens or antigen enriched fractions, and several versions of such tests are now available commercially. The most successful to date rely on flagellin or flagellin enriched antigens where the protein has been extracted under essentially non-denaturing conditions that appear to conserve specificity. This increased specificity permits the lowering diagnostic cut off values, thus also improving sensitivity. In the United Kingdom Lyme disease testing, carried out by the Lyme disease reference centres at Hereford and Southampton Public Health Laboratories, incorporates the use of

a flagellin based ELISA together with immunoblotting. Immunoblotting has been reported to be more sensitive than some ELISA tests,[2] but the principal reason for its use is that non-specific antibody can be identified and some low level ELISA false positive findings can be excluded as a result.

The wide clinical spectrum and variable course of Lyme disease make clinical recognition and diagnosis difficult. This problem is further compounded because the clinical course of Lyme disease varies in different parts of the world. Arthritis, for example, is a common chronic manifestation of Lyme disease in the USA but is less often reported in some parts of Europe. Insight into what are the principal clinical manifestations in any region, and their relative frequency, can greatly facilitate the clinician in recognising the disease symptoms and hence in referring appropriate samples for serological testing. Reporting of Lyme disease cases to the public health authorities in the UK is not compulsory and hence there is only limited information to date regarding the clinical presentations, incidence, and prevalence there.

Results

Between 1987 and 1991 15349 samples were submitted to the PHL Lyme disease reference centres for Lyme disease testing from the 51 public health laboratories and other hospital microbiology departments throughout the UK and Ireland; 170 cases of Lyme disease were confirmed. Of these, 44 (26%) had erythema migrans, and a flu-like illness was reported in 22 (13%) cases. Neurological complications were detected in 52 (31%) cases. Fourteen (8%) gave a history consistent with Lyme arthritis, of whom three had exposure to the risk of tick bites in the USA, two had received tick bites in Germany, and one in France. To date, one British case of the chronic skin manifestation acrodermatitis chronica atrophicans has been confirmed, resulting from a tick bite in the New Forest, Hampshire.

Conclusions

The clinical manifestations of Lyme disease in the UK seem similar to those reported elsewhere in mainland Europe. A significant proportion of cases detected to date have occurred in inhabitants of or visitors to the New Forest and its surrounds, Salisbury and West Wiltshire, and parts of East Anglia, Devon, and Scotland. Consequently, awareness of the disease in such areas has been heightened. Increased education among local clinicians in the New Forest as to the recognition of the early symptoms of Lyme disease has led to greater numbers of cases being treated sooner, with fewer seroconversions, and hence fewer serodiagnostically confirmed cases in this particular local population. In other areas of the UK, where Lyme disease was not previously recognised but cases have been recently identified, a coordinated public health education effort, involving the Public Health Laboratory Service, the University of Southampton Medical School, and New Forest District Council, has resulted in additional cases being found. This is further evidence to suggest that cases may be missed currently owing to limited clinical awareness in some areas of the UK. These factors underline the need for caution in interpreting the significance of changes in the number of cases diagnosed when assessing any changes in the incidence of Lyme disease in the UK.

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Diagnostic detection of *Borrelia burgdorferi* DNA by the polymerase chain reaction

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The fundamental problem in all attempts at direct detection of *Borrelia burgdorferi* is the extremely low number of organisms present in pathological lesions or body fluids from patients with Lyme borreliosis. Therefore the recent introduction of the powerful in vitro amplification of DNA by polymerase chain reaction (PCR) seemed a promising solution. Polymerase chain reaction enables a selected DNA sequence to be copied in vitro. With optimal conditions a single strand of DNA can be copied over a million fold and then subsequently detected by conventional methods, such as Southern blot hybridisation.

Polymerase chain reaction assays for detection of purified *B burgdorferi* DNA achieve a high analytical sensitivity with a lower detection limit of about 2 fg DNA corresponding to about one genome copy.[1-3] When clinical specimens artificially seeded with spirochaetes cultivated in vitro are tested a sensitivity of 20–50 spirochaetes per ml may be obtained.[4]

From a diagnostic view point the optimal target DNA sequence to be amplified should be specific for *B burgdorferi* but also highly conserved, so that DNA from all *B burgdorferi* strains would be detected. This demand is met by parts of the *B burgdorferi* flagellin gene,[4] or the 23S and 16S RNA gene,[5 6] which allow both species specific and species wide amplification. Polymerase chain reaction is as effective as immunofluorescence for the demonstration of *B burgdorferi* in infected ixodes ticks.[2] Owing to the much higher density of spirochaetes in the mid-gut of ticks compared with that in clinical specimens from patients with active Lyme borreliosis, however, the conditions are not comparable.

Data available on the diagnostic performance of PCR on clinical specimens from patients with Lyme borreliosis are so far sparse and less conclusive. In tissue from experimentally infected animals,[3] as well as in skin biopsy specimens from patients with erythema migrans and acrodermatitis chronica atrophicans,[5 7] PCR seems to achieve a diagnostic sensitivity comparable with that of culture. Thus PCR seems promising for dermatological specimens.

The reported success rates using other sample sources vary considerably. *B burgdorferi* DNA has been amplified from serum,[8] joint fluid,[9] cerebrospinal fluid (CSF),[4 6 9 10] and urine[4 11] but from only a limited number of patients with Lyme borreliosis. At present it seems that CSF, and especially urine, are the most appropriate sources of samples for PCR. When CSF samples are used the diagnostic sensitivities reported vary from 20 to 90%.[4 6 9 10] Although only few data are available on urine,[4 11] the diagnostic sensitivity on pretreatment samples is about 50% in cases of early disseminated disease. Interestingly, the diagnostic sensitivity on urine seems to increase considerably when urine is sampled three to six days after antibiotic treatment is started.[4] This phenomenon may be explained by treatment induced spirochaetal killing and then increased excretion of *B burgdorferi* DNA. Results in different reports are not comparable, however, owing to significant differences in the selection of patients. This is probably more important than differences in PCR methodology. The variable success rate of diagnostic PCR in Lyme borreliosis may be because the low number of spirochaetes in body fluids from patients is close to the detection limit of PCR, thus making the PCR assay much more sensitive to Taq polymerase inhibition by tissue components.

It is concluded that PCR on skin biopsy material might become a useful diagnostic procedure. Polymerase chain reaction has been used for the reliable detection of *B burgdorferi* DNA in CSF and urine from patients with Lyme borreliosis, but the diagnostic sensitivity remains to be defined. Future studies need to evaluate the precise performance of PCR as a diagnostic tool on a large series of patients with unequivocal Lyme borreliosis. For the time being PCR should not be accepted as the gold standard, replacing in vitro cultivation.

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Poster abstracts

Clinical manifestations and treatment

Acrodermatitis chronica atrophicans and Lyme arthritis

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Arthritis was the first reported syndrome resulting in the description of a new disease. Acrodermatitis chronica atrophicans was the first disorder described in 1883 which today belongs to Lyme borreliosis. The aim of this study was to find concomitant Lyme arthritis and acrodermatitis chronica atrophicans.

Treatment of 147 patients with Lyme arthritis and 45 patients with acrodermatitis chronica atrophicans began in 1987. In eight of the 192 patients both disorders were diagnosed, according to Steere and Asbrink. In one patient with acrodermatitis chronica atrophicans the presence of *B burgdorferi* was shown in a skin biopsy specimen by electron microscopy. Acrodermatitis chronica atrophicans developed earlier than Lyme arthritis in seven patients, but in the other subject Lyme arthritis and carpal tunnel syndrome were followed by acrodermatitis chronica atrophicans. Acrodermatitis chronica atrophicans was almost resolved eight months after antibiotic treatment, but recurrent attacks of Lyme arthritis were found in two patients despite treatment. In one patient myositis developed during the administration of ceftriaxone.

Compared with neurological involvement, the incidence of Lyme arthritis in patients with acrodermatitis chronica atrophicans is relatively low (in eight out of 45 (18%)).

Although both acrodermatitis chronica atrophicans and Lyme arthritis represent the chronic stage of Lyme borreliosis, the sequence may vary.

In our study third generation cephalosporins did not prevent further extracutaneous manifestations.

Chronic versus subacute manifestations in neuroborreliosis: role played by cranial magnetic resonance imaging

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Lyme disease is associated with a wide variety of neurological manifestations. Cranial magnetic resonance imaging is a sensitive tool in neurological diseases. We tried to define its clinical usefulness for separating chronic from subacute manifestations of neuroborreliosis.

We evaluated 39 patients (22 women, 17 men) from January 1989 to December 1992 with neurological symptoms or signs and positive serology for *Borrelia burgdorferi* (ELISA and IFA). Only subacute and chronic cases were considered.

Of the 39 patients, 14 were considered to have a chronic form (CF) of the disease; the rest were classified as subacute (SAF). The mean (SD) age was similar in both groups (49·3 (12) years for CF *v* 41·5 (14) for SAF). CF showed more frequently cognitive alterations (8/14 *v* 3/22 in SAF; p=0·08), sleep disturbances (12/14 *v* 10/24 in SAF; p=0·02), cerebellar signs (8/13 *v* 5/24; p=0·03), gait imbalance (10/14 *v* 8/25; p=0·04), and Babinski sign (7/14 *v* 2/25; p=0·09). Magnetic resonance imaging lesions were detected in 12 out of 14 patients with the CF in comparison with 10/25 in SAF cases; p=0·01. Cerebrospinal fluid IgG was higher in the CF (97 (166) *v* 281 (26) mg/l in the SAF; p=0·047), but IgG synthesis was not significantly different between the groups.

It is concluded that cognitive and sleep disturbances are more often found in the CF of neuroborreliosis. Central nervous system signs are also a criteria for the CF. Positive magnetic resonance imaging findings can help to detect chronic infection in neuroborreliosis.

Ocular manifestations in Lyme borreliosis

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Background—The large spectrum of distinct inflammatory processes that can occur within the eye is explained by the existence of 'boundary structures' which modify or limit the extension of intraocular inflammation. Ophthalmoscopy and biomicroscopy allow exact visualisation and localisation in vivo. Lyme borreliosis can induce quite distinct manifestations of intraocular inflammations.
Patients and methods—From 1986 to 1992 we observed 21 patients with intraocular inflammation in active Lyme borreliosis. Diagnostic criteria were raised IgM antibody titre which disappeared under treatment, significantly raised IgG antibody titre, or simultaneous typical extraocular manifestations.
Results—Intraocular manifestations were iridocyclitis (three patients), papillitis or neuroretinitis (six), retinal vasculitis (four), and choroiditis (eight). Extraocular manifestations were erythema migrans (four), neuroborreliosis (10), oligoarthritis (eight), and cardiac involvement (two). Two patients had only ocular inflammation. In one patient recurrent choroiditis suggested persistence of *B burgdorferi* within the choroid despite treatment. In this patient the *B burgdorferi* was cultured six months after antibiotic treatment from a joint specimen.
Conclusion—The clinical spectrum of intraocular inflammation is not pathognomonic for Lyme borreliosis but fits the spectrum of an ocular spirochaetosis, especially with regard to its variability. Ocular inflammation may be the only obvious manifestation of Lyme borreliosis. Ophthalmoscopy enables the inflammatory process in Lyme borreliosis to be visualised and followed up.

Lyme arthritis: the Somerset experience

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Although borreliosis is well documented in the United Kingdom, only one incidence of Lyme arthritis has been published. Two established cases have been treated in the above unit and a third is currently under investigation.

Patient No 1 was a 46 year old farmer with synovitis in hands, feet, and knees of two years' duration. There was no preceding tick bite. Evanescent pruritic rash was present, but there was no erythema chronicum migrans. Antibodies to *Borrelia burgdorferi* were positive. Resolution occurred after two weeks' treatment with oral penicillin. A relapse occurred three years later, which responded to four weeks' treatment with doxycycline.

Patient No 2 was a 47 year old farmer's wife. She presented with acute synovitis of the small joints of the hands, left wrist, left shoulder, and left ankle. There was no tick bite. Erythematous, macular, pruritic rash of the forearms and neck was present, but there was no erythema chronicum migrans. Antibodies to *B burgdorferi* were positive. She responded rapidly to 10 days' treatment with oral penicillin. Low grade symptoms were present for two years and she received intra-articular corticosteroid treatment for the left shoulder. Subsequent recovery was complete.

The third patient, a 43 year old male agricultural worker, presented with acute synovitis of the small joints of the hands, feet, right shoulder, left ankle, and left knee. He had a history of multiple tick bites. Rheumatoid factor was borderline positive. Antibodies to *B burgdorferi* were positive. He received intracorticosteroid to the right knee and is currently receiving tetracycline 500 mg four times a day. Follow up continues.

Lyme carditis: a clinical update

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Lyme carditis complicates infection with *Borrelia burgdorferi* in 8% of untreated subjects with erythema migrans. We evaluated seven patients with Lyme carditis during the summer of 1992, the largest group presenting in one year since the original description from this institution in 1980.

Six of the seven patients were male. The age range varied between 12 and 52 years (mean 28·5). Only two patients recalled having a tick bite, and three had a rash consistent with erythema migrans. Before the development of heart block most patients had non-specific prodomal symptoms, including fatigue, headache, fever, and arthralgias. One patient developed Lyme meningitis. Lightheadedness, palpitations, and shortness of breath were the most common presenting cardiac complaints. One subject was completely asymptomatic and was diagnosed during a routine physical examination. The patient had a new midsystolic click on cardiac auscultation. Electrocardiogram showed complete heart block in six of the seven

patients. The seventh patient had a Wenckebach rhythm. Echocardiograms obtained in five patients showed evidence of mild left ventricular dysfunction in three. Lyme titres were positive in all patients, with six having an increase of the IgM component.

All patients were treated with antibiotics (six with ceftriaxone, one with doxycycline) with resolution of complete heart block within 48 hours. All had variable degrees of first and second degree heart block for up to two weeks during treatment. Six patients initially required monitoring in an intensive care unit and one required a temporary pacemaker. All patients were in normal sinus rhythm at one month follow up. Three patients had mild residual musculoskeletal pain, which resolved within six months without further treatment.

In summary, carditis continues to be an important complication of Lyme disease. In contrast with earlier studies, most patients did not recall a tick bite or have erythema migrans. All patients responded well to antibiotics with complete resolution of disease.

Clinical and serological study of patients with Lyme borreliosis among a group of neurological inpatients

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In Liguria, an Italian region endemic for Lyme borreliosis, seropositivity for antibodies against *Borrelia burgdorferi* is not a rare finding. We examined a neurological group of patients in an attempt to differentiate patients with a causal association of neurological disease and previous spirochaetal exposure from patients with true neuroborreliosis.

One hundred and nine serum samples and 50 cerebrospinal fluid samples from neurological inpatients admitted in the years 1990–2 to the Institute of Clinical Neurology, University of Genova, and to another two divisions of neurology in the region, were tested for antibodies against *B burgdorferi* by immunofluorescence assay. Serum titres from 1/256, and cerebrospinal fluid titres from 1/5, were considered positive.

In 34 patients (31%) antibodies were detected. In 22 of them the titre was 1/256, in five 1/512, and in seven cases we observed a marked positivity (1/1024). Neuroborreliosis was diagnosed in 11/34 seropositive patients who fulfilled the diagnostic criteria of Reik (1991). Five patients had facial nerve palsy, and in two of them the palsy was bilateral and recurrent. Three patients had multiple cranial neuropathy (VII and V nerves). Two patients presented with typical Bannwarth syndrome, and one patient had a sensory-motor polyneuropathy with vasculitic changes.

These data suggest that neurological manifestations of *B burgdorferi* infection are not rare in our region, and that these are characterised by predominantly peripheral nervous system disease.

Clinical manifestations of Lyme borreliosis in an Italian endemic region

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In this study we report the clinical features of patients with Lyme borreliosis resident in Liguria, an Italian region endemic for Lyme borreliosis.

During the last eight years 1422 patients were referred to our centre because of suspected Lyme borreliosis. We confirmed the diagnosis in 195/1422 (14%) patients. The female/male ratio was 121/74 and the mean age was 46. Antibodies against *B burgdorferi* (detected by immunofluorescence assay or enzyme linked immunosorbent assay (ELISA)) were present in 163/195 (84%) patients. Thirty two (16%) patients were seronegative.

Erythema chronicum migrans (ECM) was present in 44 (22%) patients; ECM and arthritis in eight (4%); ECM and neurological manifestations in three (2%); ECM, arthritis, and cardiac disturbances in one (0·5%); ECM, arthritis, neurological, and cardiac manifestations in three (2%); arthritis and neurological symptoms in 11 (6%). Only arthritis occurred in 51 (26%) patients, and only neurological symptoms in 44 (22%); two (1%) patients had acrodermatitis chronica atrophicans, 17 (8%) had lymphadenosis benigna cuta, and one (0·5%) both ECM and lymphadenosis benigna cuta. Other skin manifestations (lichen sclerosus et atrophicans, erythema) occurred in eight (4%) patients.

Our results show that most patients, 166/195 (85%), had only one clinical manifestation of Lyme borreliosis, 23 (13%) patients had two, three (2%) patients and three, and three (2%) patients had four.

These results are in agreement with the existence of a geographical diversity of clinical manifestations of Lyme borreliosis, and we suggest that Lyme borreliosis presents in our region a different, less serious clinical pattern than in other countries.

PCR based detection of CSF *Borrelia burgdorferi* as a predictor of treatment response in Lyme neuroborreliosis

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Objective—To use polymerase chain reaction (PCR) based detection of *B burgdorferi* to predict treatment response in Lyme neuroborreliosis.

Background—Accurate diagnosis of active Lyme neuroborreliosis has been difficult, contributing to difficulty in judging treatment efficacy.

Design/methods—Forty nine patients with immunological evidence of exposure to *B burgdorferi*, and nervous system symptoms consistent with this diagnosis, underwent lumbar puncture and were treated with ceftriaxone. Previously described techniques were used to measure intrathecal antibody production, and cerebrospinal fluid was probed for *B burgdorferi*-specific DNA. Response to treatment was judged clinically, with one to four years follow up. Response was assessed before the PCR assays, and was judged as complete, partial, or failure.

Results—(a) 20 patients failed to respond; 11 were PCR negative, nine PCR positive; *(b)* 21 patients responded partially; 18 were PCR positive; *(c)* eight patients recovered completely; five were PCR negative, three PCR positive. The association between PCR positivity and treatment response was highly significant ($p=0·009$, χ^2 test).

Conclusion—PCR based detection of *B burgdorferi* genetic material predicts treatment responsiveness in patients with appropriate nervous system symptoms. False negatives may occur; treatment failures may reflect inadequate treatment or false positive results.

No serological evidence of infection with *Borrelia burgdorferi* in British patients with dilated cardiomyopathy?

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Lyme borreliosis is endemic in several regions of the United Kingdom. Cardiac disease in Lyme borreliosis is well established, and recent reports suggest it may be implicated in the pathogenesis of dilated cardiomyopathy. We tested serum samples for IgG antibodies to *Borrelia burgdorferi* from 97 consecutive patients (71 men, 26 women) with idiopathic dilated cardiomyopathy (mean age 43 years, range 12–74) referred to a tertiary centre in the United Kingdom and 77 controls (38 healthy, age and sex matched; 39 household contacts). The diagnosis of dilated cardiomyopathy was established by WHO criteria, all patients having normal coronary arteriography and no specific heart muscle diseases. Serum samples were screened by enzyme linked immunosorbent assay (ELISA) using sonicated *B burgdorferi* (strain B31) as antigen. Samples with raised antibody levels detected by this method were subsequently immunoblotted to define their antibody specificity further.

Patients had been symptomatic for a mean (SD) duration of 34 (48) months. Thirty (31%) described an acute viral illness at disease onset and 13 (13%) had either clinical (seven patients) or histological (six) evidence of myocarditis. No patients described previous symptoms compatible with Lyme borreliosis. Analysis of the ELISA data showed seven of 97 patients with dilated cardiomyopathy (7·2%) and two of 77 controls (2·6%) had antibody levels above two standard deviations of the mean of a

previously defined non-susceptible population. Immunoblot analysis, however, failed to confirm binding patterns characteristic of an anti-*B burdorferi* immune response in any of these samples.

In conclusion, no evidence has been found to implicate *B burgdorferi* in the pathogenesis of dilated cardiomyopathy in the United Kingdom. In the absence of specific symptoms or likely exposure to *B burdorferi* we would not recommend routine serological testing for Lyme borreliosis in this group of patients.

Investigations to detect *Borrelia burgdorferi* DNA in the synovial fluid of patients with an undiagnosed large joint arthritis

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Lyme borreliosis is an uncommon condition in the United Kingdom and there are few reported cases of Lyme arthritis. We have recently seen at this hospital four cases of Lyme arthritis (three following tick bites in the United Kingdom and one in the USA), suggesting it may be more common than previously thought in this country. To investigate this, synovial fluid from 40 consecutive patients with an undiagnosed large joint monarthritis or oligoarthritis presenting in the late summer and autumn was analysed, using the polymerase chain reaction (PCR), for the presence of genomic DNA encoding for the outer surface protein A (OspA) of *Borrelia burgdorferi.* A synovial biopsy sample was also available from one of the four patients with Lyme arthritis, though this had been taken after antibiotic treatment. Nested primers were used and both boiled synovial fluid (20 μl) and DNA extracted from cells pelleted by centrifugation analysed.

None of the samples, including the synovial biopsy sample, contained detectable *B burgdorferi* DNA using this method, and this may indicate that *B burgdorferi* was not the cause of the arthritis in these patients. The ability of the PCR to detect *B burgdorferi* DNA in synovial fluid has not been fully evaluated, however, and it may be an insensitive technique for diagnosing Lyme arthritis. Possibly a significantly higher yield may be obtained from the PCR of synovial tissue. *B burgdorferi* DNA was also not detected in the synovial biopsy sample taken from the patient with Lyme arthritis, possibly owing to erradication of the organism by previous antibiotic treatment as the presence of chronic arthritis (stage III Lyme borreliosis) does not necessarily reflect persistent infection.

Fibromyalgia and chronic fatigue syndrome induced by Lyme disease

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Despite antibiotic treatment for Lyme disease, persistent arthralgia, fatigue and/or subjective neurocognitive dysfunction is common. The objectives of this study were (*a*) to determine if patients with these persistent symptoms have fibromyalgia (FM) or chronic fatigue syndrome (CFS) and (*b*) to define these subjective complaints through clinical evaluation and neuropsychological testing.

Twenty three patients with persistent symptoms were compared with 23 age, sex, and education matched recovered patients. All met Centres for Disease Control criteria for Lyme disease, were enzyme linked immunosorbent assay (ELISA) positive, and had previously been treated with standard antibiotic regimens. Eleven in each group were still seropositive a mean of 5·6 years after disease onset. Of the group with persistent symptoms, seven (30%) met criteria for FM, three (13%) for CFS, and 10 (43%) had findings resembling FM or CFS, or both, but did not meet the criteria. None of the recovered patients had symptoms of FM or CFS.

Twenty two symptomatic patients complained of memory or concentration difficulties, or both, and compared with the controls had significant differences on attention/concentration subscales of the Wechsler Memory Scale-Revised (WMS-R; $p<0·01$), including digit forward ($p<0·02$) and digit backward ($p<0·01$), the Symbol Digit Modality Test (SDMT oral; $p<0·005$), and the Beck Depression Inventory (BDI; $p<0·01$); seven scored 2SD below the mean on WMS-R or on SDMT. In addition, the Symptom Checklist 90-Revised and the Minnesota Multiphasic Personality Inventory scores suggested greater hypochondriasis, hysteria, and somatisation in women and a greater degree of hysteria for men from the group with persistent symptoms ($p<0·01$). Thus despite treatment, a sequela of Lyme disease may be a post Lyme syndrome characterised by persistent arthralgia, fatigue, and neurocognitive dysfunction which is identical with or similar to FM or CFS.

Dementia in Lyme disease

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A 65 year old man was admitted to hospital with sudden onset of confusion and disorientation and diagnosed as having dementia of unknown origin. He was unable to perform even simple activities, his attention span was short, and he could not follow basic commands.

A full work up was done. Complete blood count, blood chemistry, RPR, thyroid function, antinuclear antibody test, chest radiography, lumbar puncture, computed tomography scan, and magnetic resonance imaging of the head were all normal. Neuropsychiatric and psychiatric evaluations initially reported the diffuse nature of the symptoms as either Alzheimer's disease or, more likely, a toxic metabolic process due to the rapid onset of symptoms. With further slowing of motor and mental functions, he was re-evaluated. His EEG was now abnormal, consistent with a diffuse cortical process, the slowness suggestive of subcortical dysfunction. There was no change in the patient's condition, and three months after the onset of symptoms he was transferred to a nursing home.

The patient lives in New Jersey, a highly endemic area for Lyme disease. Six weeks after transfer to a nursing home, our clinical evaluation and a reactive immunoblot for antibodies to *Borrelia burgdorferi* indicated that the patient had Lyme disease. The IgG western blot showed bands at 34, 41, 54 kilodaltons and stray bands. Intravenous antibiotic treatment with cefotaxime was started upon transfer back to the hospital. Dramatic improvement was seen, with a 90% return of motor and mental function, which continues to date. As far as we know this is the first reported case of dementia due to Lyme disease responding completely to treatment with antibiotics.

Ecology and epidemiology

Seroepidemiological study of Lyme and tick-borne encephalitis (TBE): immune status of American troops in southern Germany

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In Europe TBE virus and *B burgdorferi* are present in the same reservoir rodent (mainly *Clethrionomys glareolus*), transmitted by the same tick (*Ixodes ricinus*), and may cause partially overlapping clinical-neurological symptoms.

A study was undertaken in 1989 to assess exposure to *B burgdorferi* and TBE virus in military personnel who had arrived one to three years previously from the USA (where TBE is not prevalent) and been selected for intensive outdoor activities in southern Germany, where both Lyme disease and TBE are endemic. Paired serum samples were obtained from 511 subjects (mean age 25·9 years) before and after the summer. Antibodies against *B burgdorferi* or TBE were detected with enzyme immunoassay systems, using a *B burgdorferi* B31 strain and a TBE Neudorfl strain as respective antigens. Questionnaire data were used for epidemiological study.

IgG or IgM antibodies against *B burgdorferi* were found in one or both serum samples of 40 (7·8%) subjects. Of the 489 non-immune subjects in the first sample, 18 (3·7%) seroconverted during the study period. IgG antibodies against TBE virus were found in 37 (7·2%) subjects, but 17 of these (46%) had visited Flavivirus endemic regions. Hence these values may reflect false positives due to cross reactions. Of 489 non-immune persons, 14 (2·9%) seroconverted for TBE without any symptoms. Three subjects had antibodies both against *B burgdorferi* and TBE.

There was no significant correlation between *B burgdorferi* or TBE seropositivity or seroconversion and different forms of outdoor activities, history of tick bite or typical clinical symptoms. In the study group and in the geographic area under study a striking parallelism was shown between *B burgdorferi* and TBE seroprevalence and seroconversion rate, albeit without clinical implications. In southern Germany TBE may be warranted in the differential diagnosis of Lyme disease.

Role of host abundance in the ecology of Lyme disease

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To explore the effects of the abundance of deer and mice, the main hosts of the vector of Lyme disease in the USA, on that of deer ticks we implemented a model representing the life cycle of these vector ticks using realistic parameter estimates taken from two field sites in coastal Massachusetts. The main inputs of the model were the monthly scanning capacities and host abundance. The equation for scanning capacity has four components: tick density on hosts in a particular month, duration of activity that month, duration of tick attachment, and density of questing ticks.

In a site in which the abundance of deer remained constant and the abundance of mice varied from year to year, that of recently emerged larvae varied as well. Our studies showed that had the density of mice been held at a level that corresponded to that in a year of exceptional mouse abundance, the ticks would have thrived. Had such hosts remained scarce, tick abundance would have waned. A stable density of ticks accompanied an 'ordinary' density of mice. In the second site where deer abundance was systematically reduced to about 100 and mouse abundance fluctuated the tick population continued to grow. Our simulations suggested that the critical threshold of deer abundance in the site is eight animals.

We conclude that the abundance of deer ticks is sensitive both to the abundance of mice and of deer.

Relative contributions of transovarial and trans-stadial transmission to the maintenance of tick-borne diseases

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It is a common misconception that because of the frequently observed low coefficient of transovarial transmission of many tick-borne diseases this transmission route contributes very little to the maintenance of the disease. A simple model that includes the important epidemiological parameters of tick reproduction and mortality shows that the potential contribution of transovarial transmission is similar to that of trans-stadial transmission, even when the transmission coefficient of the former is less than 10% of that of the latter. The actual contribution will depend on the specific vector/host relationships for each tick-borne disease, and the susceptibility to the disease of the host species for each tick stage.

Ecological factors in the maintenance of *Borrelia burgdorferi* in wild animal populations

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The maintenance of *Borrelia burgdorferi* in the wild will depend on specific vector/host interactions. The vectorial capacity of the vector tick species and reservoir potential of the vertebrate species will be of critical importance. The ecological and biological factors that govern these parameters are being investigated in woodland systems in England.

A consideration of vectorial capacity must include the seasonality, feeding habits, and mortality of each tick species and their ability trans-stadially and transovarially to maintain infection. The potential of each host species to act as a reservoir for Lyme disease will depend on its ability to maintain a viable infection, the form of the tick distribution within the host population, the host seasonal dynamics, and its overall field density.

Prolonged *Ixodes dammini* attachment to humans is associated with higher incidence of *Borrelia burgdorferi* infection

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Recent studies have resulted in divergent recommendations for antibiotic prophylaxis after an *I dammini* bite. As the risk of Lyme borreliosis is low, even in endemic areas, treatment may be justified if a subgroup of persons at higher risk for infection is identified. In animal studies the risk of transmission of the spirochaete increases markedly at about 48 hours of attachment.

To determine if the risk in humans increases with longer attachment, subjects bitten by *I dammini* and not receiving antibiotics were studied prospectively. As the history is not always reliable (only 29% of our subjects could recall a precise duration of attachment) we determined duration of attachment from the scutal index (body length/scutum width), a measure of tick engorgement shown to correlate with duration of attachment. *B burgdorferi* in ticks were detected using polymerase chain reaction. Acute and six week serum samples were assayed for *B burgdorferi* antibodies by enzyme immunoassay and immunoblot. To date, among 98 subjects with evaluable *I dammini* bites, *B burgdorferi* infection developed in 1/72 (1·4%) with tick attachment <48 hours compared with in 3/26 (11·5%) with tick attachment ⩾48 hours ($p<0·05$). Manifestations were erythema migrans (two children) and asymptomatic seroconversion (one child, one adult). The high risk of infections with *I dammini* attachment of 48 hours suggests the need to study the efficacy of antibiotic prophylaxis in this subset.

Lyme disease in British horses

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A serosurvey of *Borrelia burgdorferi* infection in British horses showed infection in all parts of the United Kingdom sampled. Rates of infection were 5–10% in most areas, but were much higher (30–40%) in Thetford and New Forest areas. During the survey a case of seropositive borreliosis (from West Sussex) associated with inflammatory lameness was recorded, which responded clinically to antibiotic treatment. Subsequently, a case from Hertfordshire has been referred, with inflammatory joint signs, uveitis, and neurological/behavioural defects which terminated in euthanasia. Postmortem examinations showed encephalitis and brain swelling, with focal granulomatous changes in the meninges, eye, synovial membranes, and bronchial lymph nodes. Culture of brain and joint tissues in BSK II medium resulted in growth of spirochaetes, though *B burgdorferi* was not positively identified. Serum samples were seropositive for *B burgdorferi* antibodies (enzyme linked immunosorbent assay (ELISA)) in the period up to euthanasia. Synovial fluids were also antibody positive. Analysis of the tissues by polymerase chain reaction using primers specific for *B burgdorferi* showed infection in brain and joint tissues. This is considered to be the first horse in which current infection has been proved.

As with dog and man, it is apparent that there are far more *B burgdorferi* seropositive horses than cases of clinical Lyme disease.

Serological responses to *Borrelia burgdorferi* in British dogs

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We previously diagnosed the first case of canine Lyme disease in the United Kingdom and conducted a preliminary serosurvey of IgG responses to *B burgdorferi* in British dogs by enzyme linked immunosorbent assay (ELISA). We have since studied dogs from selected rural areas. Thirty two dogs from Thetford Forest and 67 from the New Forest areas had higher levels of antibody than 51 dogs from other rural areas ($p<0·002–0·001$). Forty seven search and rescue dogs had frequent tick exposure in many areas of the United Kingdom over periods of months to years, but none was seropositive.

Antibody responses to 11 different strains of *B burgdorferi* from Europe and North America were compared. Polyacrylamide gel electrophoresis showed antigenic differences between *B burgdorferi* strains, particularly in the size of the outer surface proteins. Individual canine serum samples, however, showed similar reactivity with different *B burgdorferi* strains by ELISA. Sequential western blot analysis of individual dogs for several months showed a time dependent expansion in the IgG response consistent with persistent infection.

We conclude that dogs in the United Kingdom are exposed to *B burgdorferi* and may harbour persistent infection without evidence of clinical disease. The development of a continuing antibody response to *B burgdorferi* in dogs may be dependent on factors other than tick exposure.

Lyme disease in Scotland: results of a serological study in sheep

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Following preliminary work which indicated the presence of antibodies to *Borrelia burgdorferi* in sheep infested with the sheep tick *Ixodes ricinus* using enzyme linked immunosorbent assay (ELISA) and western blotting techniques, a study was undertaken to assess the age at which sheep had been exposed to the organism. Blood samples were obtained from 6-8 week old lambs, hoggs (1 year old), and adult ewes, from nine sheep farms throughout Scotland, and screened by counterimmunoelectrophoresis for evidence of tick-borne fever. Samples which showed a positive reaction in this test and samples from animals infested with *Ixodes ricinus* at the time of sampling were tested using *B burgdorferi* antigens in an ELISA.

It was shown that ELISA titres obtained from ewes or lambs did not differ significantly from those for tick-free control serum samples, but that titres from hoggs (1 year old sheep) were significantly greater ($p<0.001$) than control values using analysis of variance. It was concluded that there was evidence of *B burgdorferi* in the hoggs (1 year old sheep) tested.

Engorged and semi-engorged ticks from participating farms were also examined by a polymerase chain reaction technique for evidence of *B burgdorferi* infection, and preliminary results indicate that 4·2% of ticks examined were infected and that positive samples were obtained from 42·9% of participating farms. It is proposed to extend the sample of the serosurvey to measure more accurately the potential reservoir of infection within the Scottish sheep population.

Comparison of vertebrate hosts and habitat or vegetation as sources of *Borrelia burgdorferi* polymerase chain reaction and ticks

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During 1990–2 a network of British tick collectors donated ticks to the Institute of Virology and Environmental Microbiology for examination by polymerase chain reaction using primers specific for *Borrelia burgdorferi*. The network extends to 35 organisations or members of the public and includes >100 contributors. Ticks were either collected by 'questing', by blanket dragging the vegetation, or by 'feeding', by removal from vertebrate hosts. The vertebrates sampled ranged from wild mammals and birds, domestic pets, farm animals, culled deer, and humans. The 255 donations were divided into two groups: 42% derived from hosts and 58% from habitat. Of these donations, 64% in total were positive. Sixty five per cent of the host associated samples and 67% of the habitat derived samples were positive.

The host species from which positive polymerase chain reaction results came were: deer (red deer, roe deer, muntjac deer), dog, sheep, human, horse, pheasant, cat, squirrel, bank vole, and field mouse. A wide range of ecosystems was represented. Also there was a variety of vertebrate hosts which yielded positive results. All species examined gave some positive results. Thus animals are efficient and effective sentinels for ticks, sampling the various locations that they inhabitat.

These results show that the Lyme disease spirochaete, *B burgdorferi* is present and a potential risk to human health in various geographical areas of the United Kingdom.

Breaking the cycle: environmental control of borrelia

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An attempt was made to assess relative levels of larval, nymphal, and adult stages in tick (*Ixodes ricinus*) populations on different upland vegetation communities in the north (North York Moors) and the south west (Quantock Hills) of England. Sites dominated by bracken (*Pteridium aquilinum*), bilberry/whortleberry (*Vaccinium myrtilus*), and heathers (*Ericacea*) were surveyed in both biogeographical regions, and results on tick populations for the first period of the study are available.

Initial results indicate differing levels, survival, and inter-instar transfer rates in tick populations of these particular vegetation communities. Intervention (burning, cutting, and spraying) on bracken dominated sites was carried out to evaluate the impact of selective management techniques on tick numbers and therefore the long term survival and development prospects of the population.

Ticks collected were assayed for *Borrelia burgdorferi* and small mammal trapping used to investigate possible relationships between ixodes populations and potential host reservoirs of the spirochaete.

The overall aim of the research is to target land management regimens, which, if introduced into tick infested upland areas, will aid in the prevention/reduction of Lyme disease transmission to human groups at risk.

Seroconversion for Lyme borreliosis and tick-borne encephalitis among Dutch military personnel

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Tick-borne encephalitis (TBE), also known as Früh-Sommer meningoencephalitis, is a viral disease endemic in many parts of Europe (in particular central Europe). Tick-borne encephalitis and Lyme borreliosis are the most important diseases transmitted by ticks in Europe.

We assessed the rate of seroconversion for Lyme borreliosis and TBE among 932 Dutch military personnel (mean age 20·6) using paired serum samples. One sample was taken immediately after entering the service in January and March of 1991 and the second 9–12 months later. The samples were tested for IgG and IgM antibodies against *Borrelia burgdorferi* with a flagel enriched enzyme linked immunosorbent assay (ELISA), and for TBE antibodies with a commercially available Immunozym Früh-Sommer meningoencephalitis IgG ELISA assay, confirmed with an HI test.

Of the 932 subjects, 14 (1·5%) seroconverted for IgM or IgG antibodies, or both, against *B burgdorferi*. By means of the western blot technique we found supportive evidence in nine of these 14 cases. None of them had a history of clinical manifestations of Lyme borreliosis, except one who had noticed an erythema migrans.

Antibodies against the TBE virus were present in one or both samples of 36 (2·6%) subjects. Seroconversion was found in 10 subjects (1·1%). No cases of clinical TBE were reported. There was no association between *B burgdorferi* and TBE seropositivity or between seroconversion for Lyme borreliosis or TBE and a history of tick bites. In conclusion, seroconversion for Lyme borreliosis and TBE occurs among Dutch military personnel, but the clinical incidence of both diseases is low.

Polymerase chain reaction in the direct detection of *Borrelia burgdorferi* from *Ixodes dammini*

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Detection of *Borrelia burgdorferi* from *Ixodes dammini* (deer ticks) was accomplished by the polymerase chain reaction (PCR). This assay involves a simple tick extraction procedure, amplification of *B burgdorferi* 16s rDNA gene sequences, followed by detection of the amplified material (amplicon) using an enzymatic based microwell plate hybridisation assay.

One hundred and sixty five field collected ticks from northern New Jersey, USA, were screened for the presence of spirochaetes with both dark field microscopy (DFM) and PCR. PCR identified 29 infected ticks, resulting in 17·6% infectivity. Twenty seven of these specimens were positive by DFM. One sample was positive by DFM while PCR negative. Three discrepancies were found, resulting in 98·8% concordance between DFM and PCR.

Our PCR procedure has proved to be a useful, accurate, and time saving technique for the detection of *B burgdorferi* in ticks. The assay takes about four hours to evaluate up to 92 ticks, and does not require the use of live ticks as with DFM and direct fluorescent antibody.

Borrelia burgdorferi shown by polymerase chain reaction from a skin biopsy specimen after a fly bite

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A previously healthy, 41 year old doctor after jogging on 17 August 1992 was bitten by a deerfly. The patient recalled no tick bites in his life. An erythema migrans-like lesion developed around the bite area in the right lower leg slowly over two weeks. No other symptoms developed. Examination on 2 September disclosed 5 × 12 cm homogeneous erythema.

A histological biopsy specimen from the centre of the erythema was cut serially: no pieces from the fly's stinger were found, but there was heavy lymphoid infiltrate consistent with insect bite reaction. Histological sections from the border of the lesion showed the characteristics of erythema migrans. DNA extraction was made from another specimen taken from the border of the erythema. *Borrelia burgdorferi* DNA could be detected by a polymerase chain reaction.

Serum antibodies to *B burgdorferi* were measured from serum samples obtained on 2 September, 22 September 1992, and 7 January 1993. Seroconversion against the flagellar antigen of *B burgdorferi* was detected in the second serum. The amount of antibodies was again negative in the third serum.

Erythema migrans disappeared completely in one week during treatment with amoxycillin 1 g twice a day. The antimicrobial drug treatment was continued for 25 days. No other symptoms of borreliosis have appeared during follow up of five months.

This patient's case further supports the observation reported by Luger (*N Engl J Med* 1990;**322**:1752) that Lyme borreliosis can be transmitted by a biting fly, and that a very short attachment of the fly is enough for the transmission.

An equine case of Lyme disease: lesions found at postmortem examination

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A postmortem examination was conducted on an 8 year old thoroughbred mare suspected on clinical grounds of having Lyme disease. Serum and synovial fluid samples had positive enzyme linked immunosorbent assay (ELISA) titres to *Borrelia burgdorferi*. Terminally the mare had become uncontrollably excited, necessitating euthanasia by shooting. Postmortem lesions were characterised by small focal granulomata with caseous centres; giant cells were often present. These lesions were present in the hypertrophic synovial membranes of several distended joints and tendon sheaths, in bronchial lymph nodes, on the epicardium, and in the meninges (associated with focal meningoencephalitis). Also present were mild inflammatory changes in the eye, pleura, and kidney. No borrelia organisms were seen using silver stains. Diagnosis of *B burgdorferi* infection was confirmed by a polymerase chain reaction technique on tissue culture supernatants.

Epidemiology of Lyme disease in Italy

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In 1983 Lyme disease was first described in Italy and was reported, especially, in northern regions. Endemic areas were Friuli-Venezia Giulia provinces with more than 600 cases reported in the last seven years, Liguria provinces with over 160 cases described, and Trentino Alto Adige with more than 30 cases. The first national congress took place in Trieste on 27–28 November 1992 organised by the Italian Lyme Disease Group. The congress allowed a better evaluation of Lyme disease epidemiology in Italy. The disease was reported in the provinces of Venetia (Belluno, Rovigo, Treviso), Lombardy (Brescia, Lecco, Milan, Pavia), Emilia-Romagna (Parma, Faenza), Tuscany (Pistoia, Siena), Latium (Rome), Apulia (Bari), Campania (Naples), and Sardinia (Sassari).

In the provinces where Lyme disease is endemic borrelial antibody titres were detected in patients, groups at risk, the healthy population, and animals and *Borrelia burgdorferi* isolated in BSK from tick and patients (Trieste) or from tick only (Bolzano and Pistoia).

In the provinces where only isolated cases were found specific antibody titres were determined in patients with supposed Lyme disease and in groups at risk.

Lyme borreliosis in Australia

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In Australia patients with clinical symptoms that fulfil the case definition of Lyme borreliosis are being seen in increasing numbers. Most patients have chronic neurological or rheumatological symptoms, live in heavily tick infested coastal regions of the country, and are frequently bitten by ticks. Serological confirmation of Lyme borreliosis by immunofluorescence assay or enzyme linked immunosorbent assay (ELISA) tests based on B31 have been disappointing, with positivity rates of only 2–3%.

Attempts to isolate *Borrelia burgdorferi*, either from patients or candidate tick vectors, so far have been unsuccessful but fastidious; slow growing borreliae are common in Australian ixodes ticks and may be responsible for indigenous disease.

By immunoblotting, a significant number of patients are positive for antibody to the burgdorferi specific antigens OspA and OspB. A larger number are positive for the 41K flagellin polypeptide. OspA positive patients are positive when European strains of *B burgdorferi* are used as antigen, but are usually negative against B31 OspA.

The results suggest an indigenous Lyme borreliosis in Australia, whose cause may be related to but different from classical *B burgdorferi*. The usefulness of Eurasian borreliae as antigen, and the search for antibody to other immunodominant borreliae antigens, is continuing.

Biology and immunopathogenesis

Molecular and immunological characterisation of a new polymorphic lipoprotein of _Borrelia burgdorferi_

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We cloned, expressed, and biochemically characterised a new polymorphic *B burgdorferi* lipoprotein recognised by monoclonal antibody LA7. Sequence analysis showed an open reading frame encoding a 21 866 dalton protein (IpLA7). Comparison with other proteins indicated sequence similarity of IpLA7 signal peptides with those of other prokaryotic lipoproteins, including the immunodominant *B burgdorferi* outer surface proteins OspA, OspB, OspC, and OspD. Both natural and recombinant IpLA7 could be biosynthetically labelled with [^{3}H]palmitate.

Upon solubilisation of intact *B burgdorferi* with the non-ionic detergent Triton X-114, IpLA7 was together with other lipoproteins selectively extracted into the detergent phase. Indirect immunolabelling studies indicated that the epitope recognised by monoclonal antibody LA7 is mainly localised to the periplasmic space. Two dimensional gel electrophoresis and immunoblotting confirmed the calculated acidic pI of 5·7 for IpLA7.

The LA7 gene is shown to be species specific and to be located on the linear chromosome of *B burgdorferi*. Analysis of 40 individual spirochaetal isolates revealed a considerable genotypic heterogeneity of LA7 corresponding to that previously found for OspA. Unlike other chromosome derived genes (fla, hsp60, hsp70) which show only limited genotypic variability the LA7 gene represents the first example of a *B burgdorferi* chromosomal gene with extensive polymorphism. Native and recombinant IpLA7 were recognised by immune serum samples from infected mice and may also prove useful as an additional marker for serodiagnosis of Lyme disease.

Chemotaxonomy of borrelia species

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Fatty acid profiles have been shown to be a stable phenotypic property of a number of bacterial species.The fatty acid profile can be obtained by gas-liquid chromatography of fatty acid methyl ester extracts from the bacteria.

Using fatty acid methyl ester profiles we have shown interspecies differentiation of spirochaetes, and we now propose that intraspecies differentiation of borrelia species is possible using this phenotypic method. The delineation of *B burgdorferi* into *B burgdorferi* sensu stricto, *B garinii*, and group VS 461 is maintained. In addition, isolates of *B hermsii* form a separate group, and *B turicatae* and *B parkeri* cluster together. The *B garinii* group contains the reference isolate, 20047, and a number of Swiss isolates. Group VS 461 contains mostly European isolates, whereas *B burgdorferi* sensu stricto contains the reference isolate, B31, and several other American isolates.

Restriction polymorphism of polymerase chain reaction products as an epidemiological and diagnostic tool for characterisation of borrelia associated with Lyme borreliosis

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Three genospecies of borrelia associated with Lyme borreliosis have been delineated on the basis of DNA homology.[1] Considering the original organisation of rRNA genes in borrelia,[2] we used the published sequence to design polymerase chain reaction primers in the 3' end of the 5S rRNA gene (rrf) and in the 5' end of the 23S rRNA gene (rrl). The uniqueness of these tandemly repeated 23S-5S rRNA genes among previously studied bacteria made these primers suitable for specific diagnosis of Lyme borreliosis despite primer unspecificity. The 254 base pair long amplification product corresponded to a variable sequence between two conserved structures. DraI restriction site polymorphism of the amplified sequence provided a useful tool for identification of the defined genospecies and characterisation of unclassified strains. This polymorphism could also be used for epidemiological purposes.

1 Baranton G, Postic D, Saint Girons I, *et al.* Delineation of Borrelia burgdorferi sensu stricto, Borrelia garinii sp. nov., and group VS461 associated with Lyme borreliosis. *Int J Syst Bacteriol* 1992; **42:** 378–83.
2 Schwartz J J, Gazumyan A, Schwartz I. rRNA gene organization in the Lyme disease spirochete, Borrelia burgdorferi. *J Bacteriol* 1992; **174:** 3757–65.

Investigation of genetic changes associated with attenuation of *Borrelia burgdorferi* by in vitro culture

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Infectivity of *Borrelia burgdorferi* is lost during in vitro cultivation in Barbour-Stonner-Kelly (BSK) medium. We are currently performing studies to identify and characterise genetic changes associated with attenuation.

Two low passage strains—a German tick isolate from *Ixodes ricinus*, and an American skin biopsy isolate, NCH-1—were serially passaged 20 times in BSK II medium. Evaluation of infectivity was performed at every fifth passage (P.5, P.10, P.15, and P.20) using a BALB/c mouse model to define an endpoint of infectivity during in vitro passage. The results of culture and polymerase chain reaction of mouse urinary bladder and cardiac tissue will be presented.

In parallel with these studies we characterised: (*a*) the plasmid profiles of P.5, P.10, P.15, P.20 by pulsed field and two dimensional gel electrophoresis; (*b*) the whole cell protein profiles of these passages by polyacrylamide gel electrophoresis.

Restriction fragment length polymorphisms using rare cutting enzymes and hybridisation experiments using rRNA, OspA and telomeric probes have also been undertaken. No gross genomic differences were detected, though rRNA restriction patterns delineated the two isolates into different genospecies. Studies to compare genomic libraries and individual plasmids from each passage are underway.

OspB sequence variation of *Borrelia burgdorferi* within islands in the Gulf of Maine

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Although many investigators are looking at Osp variation throughout the USA and in Europe, local genic variation within a spirochaetal population has not been considered. We studied twelve borrelia isolates from islands in the Gulf of Maine and two isolates from the Maine coastline. Lyme disease foci on the islands involve different mammalian hosts and are presumably more isolated from gene flow than mainland populations. Our expectation was that these isolates would contain single strains, possibly adapted to the unique set of conditions on the islands.

After sequencing the OspB gene of these isolates we found an unexpectedly high level of diversity. The range of variation was found to be between zero and nine nucleotide changes (which translated to a maximum number of six amino acid changes). The average pairwise difference in the OspB gene was 0·32%. Five strains (defined as containing at least one amino acid substitution) were found among the nine isolates from Monhegan Island. One of these strains was found among three larvae taken from the same rat at the same time. The two mainland strains were identical to two strains found on Monhegan Island. Isle au Haut itself contained two different strains out of three isolates and one was identical to a Monhegan Island strain. Birds may play a part in strain dispersion. High diversity of *B burgdorferi* strains may be an important aspect of the ecology of Lyme disease and may have a role in determining the abundance of this spirochaete.

Lyme disease in an experimental cat model

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Despite broad species susceptibility from dogs to horses, attempts experimentally to reproduce Lyme disease in laboratory cats have been disappointing. We examined the susceptibility or development of Lyme borreliosis, or both, in experimental cats under category 2 containment. Three groups of cats were injected with 10^6 live spirochaetes in a single intradermal site. The three strains of *Borrelia burgdorferi* (Bb) used were: Bb31 reference strain, Bb1579 strain isolated from an *Ambyloma americanum* tick, and Bb532 strain isolated from a pool of five cat fleas. Cats were examined daily, bled biweekly, and one cat per group killed monthly for histopathological study.

Initial results indicate that all three strains of *B borreliosis* are immunogenic in cats and caused some lesions which are characteristic of Lyme borreliosis. However, spirochaetes could not be recovered from ticks or cat fleas that fed upon these experimentally infected cats. Differential white blood cell counts indicated that the cats were infected. Histological data indicated that multiple organs were infected in test cats, including regional lymph nodes, lungs, and liver. The presence of whole intact or fragmented spirochaetes at the site of lesion(s) was determined using Steiner's silver stain for spirochaetes in tissue sections.

Lyme disease in an experimental mouse model

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We developed an in depth mouse model of Lyme borreliosis and analysed the generation of humoral and cellular responses and the pathogenesis after experimental inocculation of viable spirochaetes. For this study we used three strains of *Borrelia burgdorferi*: *B burgdorferi* 31 reference strain, *B burgdorferi* 1579 strain isolated from an *Ambyloma americanum* (Lone Star) tick, and *B burgdorferi* 532 strain isolated from a pool of five cat fleas. We showed that all three strains were highly virulent and capable of causing Lyme disease in all the B10 H-2 congenic and recombinant mouse strains. Results

indicate that it is possible to generate dermatological, pulmonary, splenic, hepatic, and cardiac lesions, in addition to arthritis, in these mice. These lesions were generated using many different routes of inoculation, including intraperitoneal, subcutaneous, intramuscular, and intradermal. The presence of whole intact or fragmented spirochaetes at the site of lesion(s) was determined by either Warthin-Starry or Steiner's silver stain for spirochaetes in tissue sections. Using B10 congenic and recombinant strains, we examined the specificity of genes within the H-2 complex responsible for susceptibility or resistance, or both, to borreliosis caused by these different isolates. We found that the genetic control of susceptibility maps to both the class I and class II genes of the major histocompatibility complex.

Borrelia burgdorferi detected in the blood, synovium, and skin of patients with Lyme arthritis

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From a selected group of 40 of 163 patients with Lyme arthritis, 20 samples of synovium, 28 of blood, and five of skin were examined for presence of *Borrelia burgdorferi*. Spirochaetes were seen by electron microscope in two specimens of synovium, six of blood, and one of skin. All attempts at cultivation of these organisms failed. All patients in whom borreliae were detected had chronic arthritis lasting for one to five years. In one patient arthritis was so severe that *x* ray examination showed destructive changes of the sternoclavicular joint at the one year follow up. These findings emphasised the major role of *B burgdorferi* in developing destructive arthritis. In one patient who developed arthritis, together with the skin lesion acrodermatitis chronica atrophicans, borreliae were found only in a sample of the skin.

Demonstrating the presence of spirochaetes may facilitate a better understanding of the pathogenesis and natural history of Lyme disease.

Sectional uptake and cytosolic processing of *Borrelia burgdorferi* by human phagocytes

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The spirochaete *Borrelia burgdorferi*, the causative agent of Lyme disease, is a long organism, so that its uptake and processing may require special means by the phagocytes. We investigated the phagocytosis of *B burgdorferi* by human monocytes, macrophages, and neutrophils with both electron and confocal light microscopy. Our results show that the spirochaetes are not ingested as entire organisms but rather section by section. Extracellular and intracellular sections may follow each other on the same spirochaete. Thus the same spirochaete may enter and leave one phagocyte several times. Alternatively, different sections of one spirochaete may be engulfed independently by different phagocytes. Following the uptake via coiling phagocytosis, the internalised sections of the *B burgdorferi* cells are released into the cytosol, but remain enveloped by a dense actin cortex. This envelope may hinder the access of vesicular cell organelles, as the spirochaetes disintegrate without apparent participation of lysosomes. It is concluded that both the sectional uptake and the cytosolic, non-lysosomal processing of the spirochaetes may contribute to the pathogenesis of Lyme disease.

Protective activity of antisera against isolates of *Borrelia burgdorferi* from various geographical origins

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Antisera from rabbits immunised with two Japanese strains of *Borrelia burgdorferi*, HP3 an isolate from *Ixodes persulcatus* and HO14 an isolate from *I ovatus*, or the European strain P/Bi isolated from human cerebrospinal fluid did not protect hamsters from challenge with the infectious strain 297, a North American isolate from patient cerebrospinal fluid. Antisera to strains 297 and B31, a North American isolate from *I dammini*, however, provided a protective effect to challenge with strain 297. Immune mice serum samples in the presence of homologous *B burgdorferi* antigen induced the production of oxygen intermediates from mouse peritoneal exudate cells. Heterologous *B burgdorferi* antigen had no effect. These results suggest that antigenic properties of Japanese strains are different from those of North American and European isolates.

Multiple sequence alignment of the osp operons of different *Borrelia burgdorferi* isolates

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OspA and OspB are major outer membrane proteins of *B burgdorferi*. These proteins are strongly antigenic, and a large number of patients with clinical manifestations of Lyme disease develop antibodies reactive with them. Biochemical and immunological studies indicated pronounced differences in OspA and OspB proteins between *B burgdorferi* isolates from North America and Europe.

To learn more about the variation of OspA and OspB, and for future evaluation of the gene products, we cloned and sequenced the entire osp operon of the European strain B29. We compared the deduced amino acid sequence of the cloned operon with that of other *B burgdorferi* strains. Our studies clearly indicated that OspA proteins show a higher degree of similarity than OspB proteins: the OspA of B29 shares 80·7% and 85·3% identity with the American strains B31 and 25015; 81% identity with the European strain ACAI, 92·3% and even 98·2% identity with the European strains IP90 and GOE2. Comparison of the amino acid sequences of OspB showed that B29 exhibits 62·7% identity with B31, 62·8% and 66·3% identity with ACAI and IP90.

In view of the variability of these two proteins in *B burgdorferi* these data are of relevance for all those who work on OspA and OspB as a possible vaccine candidate.

Molecular cloning of a linear plasmid located gene encoding a *Borrelia burgdorferi* B29 specific 27 kilodalton surface lipoprotein

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Antiserum of a patient with cutaneous manifestations of Lyme borreliosis was used to isolate the gene encoding a 27 kilodalton protein antigen (P27) of the European *Borrelia burgdorferi* strain B29 from a λ-gt11 expression library. It encodes a basic protein of 248 amino acids with a typical prokaryotic leader sequence at the 5'-terminus of the coding sequence. Pulsed field gel electrophoresis and subsequent Southern blot analysis showed that the cloned gene is located on a 55 kb linear plasmid. The same plasmid of strain B29 also contains the osp operon encoding the major surface proteins OspA and OspB. The p27 gene did not hybridise with linear plasmids of the American *B burgdorferi* strain B31. Northern and western blot analysis confirmed the strain specifity of this protein. Biochemical investigations showed that p27 is a surface exposed lipoprotein. The cloned gene was overexpressed in *Escherichia coli*, and the purified recombinant protein reacted with several antisera from patients with cutaneous manifestations of Lyme borreliosis.

Activation and killing of *Borrelia burgdorferi* by leucocytes in vitro

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Uptake of *B burgdorferi* by professional phagocytes has been described by many authors. The reported data, however, failed to show the efficacy of killing on intracellular borreliae and the biochemical mechanisms involved. We studied the interactions between polymorphonuclear leucocytes and one strain of *B burgdorferi*. Cultural studies by limiting dilution showed that a remarkable killing effect was detected only after opsonisation by antibodies and complement. More data on activation of leucocytes and killing were obtained by flow cytometry.

Plasmid analysis of *Borrelia burgdorferi*, Chinese isolates

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Plasmid composition of 18 *B burgdorferi* isolates from China was analysed by low percentage agarose gel electrophoresis, and genomes of several *B burgdorferi* isolates were characterised by REA and DNA hybridisation using labelled *B burgdorferi* genomes. Each isolate had three to eight discernible plasmids, ranging in size from 60 to 12 kb. Several different restriction and homology patterns were seen among these isolates; Chinese isolates appear to have a more genotypic heterogeneity than North American and European isolates.

Close correlations were found between the results of plasmid profiles and whole cell DNA restriction endonuclease as well as DNA hybridisation. It was concluded that plasmid profile analysis may be a reliable and accurate method for identifying strains of the Lyme disease agent.

Repeated DNA sequences on circular and linear plasmids of *Borrelia burgdorferi* sensu lato

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We cloned a 3·8 kb *Eco*RI fragment from the 29 kb circular plasmid of the *Borrelia burgdorferi* sensu stricto type strain B31, which hybridised to several additional restriction fragments of the 29 kb circular (1·4, 6·4, 7·0 kb *Eco*RI) and the 50 kb linear plasmid (4·5 kb *Hin*dIII) of this strain. They were also cloned and their cross hybridising segments identified on subclones.

Partial DNA sequence analysis disclosed a region (I) displaying >85% homology at least 700 bp long, and (II) containing the 5' end of a putative open reading frame longer than 600 bp. Whether this sequence is being transcribed and yields a polypeptide is still under investigation.

Signals with linear and circular plasmids of other Swiss and American isolates assigned to the *B burgdorferi* sensu stricto, *B garinii* species or the VS 461 group were detected as well. No hybridisation, however, was seen with *B coriaceae, B hermsii, B parkeri,* and *B turicatae* genomic DNA. Thus the repeated sequence seems to be specific for *B burgdorferi* sensu lato and therefore may be useful in nucleic acid based diagnostics of Lyme disease.

Typing non-cultivatable *Borrelia burgdorferi* by asymmetric polymerase chain reaction and sequencing

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Although not reaching the endemic proportions of north east America, Lyme disease in Britain affects outdoor workers and recreationalists. In Scotland, among a high risk group of nature conservancy workers, 19·0% were IgG seropositive for *Borrelia burgdorferi*. Although high carriage rates of *B burgdorferi* in *Ixodes ricinus* ticks have been shown, no isolate has been obtained in culture.

We are typing these non-cultivatable *B burgdorferi* by sequencing polymerase chain reaction products using asymmetric polymerase chain reaction, and comparing the results with the sequences of *B burgdorferi* from Europe and America. Also included in this study will be non-culturable strains from Ireland and England. The information may provide a clue as to why *B burgdorferi* are so difficult to isolate in these Isles, and aid serodiagnosis in the region.

Differentiation of *Borrelia burgdorferi* isolates from ticks and humans by different monoclonal antibodies in immunofluorescence

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The application of monoclonal antibodies is very useful for identification of spirochaetes isolated from ticks and patients. According to such investigation with monoclonal antibodies, several serogroups were proposed for *Borrelia burgdorferi*. In this study an IgM monoclonal antibody was tested additionally to the IgG monoclonal antibodies. Altogether 21 isolates (18 from ticks, three from patients) were tested by indirect immunofluorescence test (IFT) using three IgG monoclonal antibodies (H9724, H5332, H3TS) and one IgM monoclonal antibody (11G1). While H9724 is specific for the genus *Borrelia*, the other monoclonal antibodies are specific for the species *B burgdorferi*. All 21 strains reacted with H9724.

According to the IgG monoclonal antibody reaction pattern of BARBOUR resulting in the formation of three serogroups, all strains tested were classified as follows: type I six strains, type II 14 strains, and type III one strain. From the total collection of strains, only six (five from ticks, one from a patient) reacted with IgM monoclonal antibody, of which three belonged to the IgG monoclonal antibody type I and another three to the IgG monoclonal antibody type II group. The frequently observed antigenic variability is of importance for the differentiation of *B burgdorferi* strains during epidemiological studies.

Diagnosis

Western blotting improves detection of specific IgM in serum of patients with erythema migrans

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Patients with erythema migrans often have a poor antibody response. To improve diagnostic sensitivity three immunoassays were used to detect specific IgM in 62 serum samples from patients with erythema migrans: IgM western blotting and a μ capture enzyme linked immunosorbent assay (ELISA), which both used a sonicate of American *Borrelia burgdorferi* isolate B31 as antigen, and an indirect ELISA based on flagella and pC protein, prepared from German *B burgdorferi* isolate pKo (AMC ELISA). Western blotting detected IgM, which reacted mainly against the flagellar antigen, in 33 serum samples. Twenty three samples were positive by μ capture ELISA, and the AMC ELISA detected IgM in 17 serum samples. Eight serum samples were positive by western blotting only. Two samples were positive only in the AMC ELISA, probably owing to a reaction with the pC antigen which is poorly expressed in B31.

It is concluded that western blotting is the most sensitive assay for the detection of IgM. Sensitivity may be enhanced further when a European isolate is used as antigen.

Isolation of *B burgdorferi* from clinically normal skin six months after disappearance of erythema migrans

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A 21 year old soldier who participated in a seroepidemiologic study on Lyme borreliosis was examined for clinical manifestations of this disease. The subject seroconverted for IgG antibodies against *B burgdorferi* measured by a flagel enriched enzyme linked immunosorbent assay (ELISA) and by immunoblotting. He recalled an erythematous skin lesion about 13 cm in diameter on his right calf. The lesion had disappeared after one month without treatment of antibiotics. A tick bite was not remembered. Dermatological, general physical, and neurological findings were normal. Six months later a punch biopsy specimen was taken from the normal appearing skin of the right as well as the left calf. After incubation in modified Kelly's medium for 14 days *B burgdorferi* was cultured from the skin biopsy specimen taken at the site of the former skin lesion. The punch biopsy of the left calf yielded no spirochaetes. The subject was treated with antibiotics; after treatment *B burgdorferi* could not be cultured from a control biopsy sample.

We conclude that *B burgdorferi* can be isolated from clinically normal appearing skin at the site of a former erythema migrans from a subject without manifestations of disease.

Detection of *Borrelia burgdorferi* DNA in clinical samples using a nested polymerase chain reaction

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A nested polymerase chain reaction has been developed to detect *Borrelia burgdorferi* DNA in clinical samples. The primers used are

complementary to conserved sequences in the OspA operon so that *B burgdorferi* sensu stricto, *B garinii*, and group VS 461 strains are detected but not distinguished. The reaction is specific for the three Lyme disease spirochaetes, whereas *B duttonii*, *Treponema denticola*, and other commonly isolated bacteria are not amplified. The sensitivity of the reaction is 1 fg of DNA (equivalent to one molecule of DNA) when products are visualised by agarose gel electrophoresis.

B burgdorferi DNA has been successfully detected in cerebrospinal fluid, serum, urine, and tissue samples. We have also shown the importance of alcohol preservation of samples to prevent the degradation of DNA in transit. An extraction method incorporating a guanidine thiocyanate lysis buffer and diatoms has been developed to adsorb nucleic acids in fluid samples. DNA is extracted from tissues samples by standard methods. Contamination is prevented by the use of positive displacement pipettes and separating areas for sample preparation, polymerase chain reaction, and electrophoresis of products. All samples are tested in duplicate, with a negative control and a reaction control, using human transferrin gene primers.

Isolation of *Borrelia burgdorferi* from normal cerebrospinal fluid in seronegative patients with erythema migrans and peripheral facial palsy

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Three male patients in whom *Borrelia burgdorferi* was isolated from their cerebrospinal fluid (CSF) are presented. Basic data are shown in the table.

Both patients 1 and 2 with erythema migrans (EM) had a typical skin lesion. One of them (No 1) reported mild systemic symptoms (fatigue, intermittent headache, low fever) while the other was completely asymptomatic. They had no signs of central nervous system disease. In the third patient peripheral facial palsy (PFP) was the sole clinical manifestation of the disease. In all three patients CSF biochemical values were in the normal range and *B burgdorferi* antibody titres in serum and CSF were negative. In all of them, however, *B burgdorferi* was isolated from the CSF.

Conclusions—In patients with EM dissemination of borreliae to the central nervous system may occur early in the course of illness and may be asymptomatic. The absence of borrelial antibodies and normal biochemical CSF findings in patients with EM and PFP do not guarantee against the presence of the causative agent in CSF. This finding may explain treatment failures with the oral antibiotics usually recommended in some patients with EM and PFP and point to the use of those antibiotics which would achieve high enough levels in CSF.

Patient data

Data	*Patients*		
	1	*2*	*3*
Age (years)	31	39	15
LB* manifestation	EM*	EM	PFP*
Other symptoms			
Local	–	–	–
Systemic	+	–	–
Tick bite	+	+	–
Time from bite to onset (days)	7	35	–
Time from onset to spinal tab (days)	4	11	9

*LB=Lyme borreliosis; EM=erythema migrans; PFP=peripheral facial palsy.

Comparative evaluation of commercially available diagnostic tests for detection of Lyme borreliosis

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The aim of the study was to evaluate the diagnostic significance of various serological methods in the routine laboratory management of antibody detection against *Borrelia burgdorferi*. Eighty preselected serum samples were analysed by the following techniques: western blot, immunofluorescence, indirect haemagglutination, ELFA, and various enzyme linked immunosorbent assays (ELISAs). Clinical data of 54 samples were collected retrospectively by a questionnaire. A combination of western blot results and clinical data served as the reference to assess the diagnostic significance of all other techniques tested.

The combined results allowed the following conclusions: (*a*) Levels of antibodies measured by immunofluorescence and various ELISAs correlated highly among each other, with one exception (recombinant p39 ELISA). (*b*) Astoundingly, no correlation was obtained between the presence of a p39 band in the western blot and the result of the recombinant p39 ELISA. (*c*) The sensitivity and specificity of individual tests differed depending on the cut off point of the test, but, in addition, differences in sensitivity/specificity curves were found. (*d*) Further criteria, like ease of use (for example, automation) and costs, have to be considered in choosing a routine laboratory test for Lyme borreliosis.

A comparative study of cross reactivity in Lyme borreliosis serology

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Syphilis, autoimmune diseases, and leptospirosis have been reported to be a source of false positive results in Lyme borreliosis serology. The aim of our study was to compare an immunofluorescence assay (IFA) and an enzyme linked immunosorbent assay (ELISA) (IgG and IgM) in their ability to cross react in routine laboratory diagnosis of Lyme borreliosis. Three groups of positive consecutive serum samples were tested. One hundred and two serum samples of the group with syphilis were positive in RPR or *Treponema pallidum* haemagglutination test or fluorescent treponeme antibody absorption test. One hundred serum samples of the group with leptospirosis were positive in a microagglutination test or ELISA. One hundred serum samples of the group with autoimmune diseases were positive in one of the following tests: antinuclear antibody, antitissue antibodies, anticardiolipin antibody, rheumatoid factor.

In IFA only the group with syphilis presented positive results (5%). In ELISA IgM 11% of the group with syphilis, 9% of the group with autoimmune diseases, and 1% of the group with leptospirosis were positive. The ELISA IgG technique showed a higher amount of cross reactions (41% in the group with syphilis, 20% in the group with autoimmune diseases, and 86% in the group with leptospirosis).

According to this qualitative approach, IFA seems to be more specific than ELISA. But the intensity of ELISA cross reactions remained in a low range, except for ELISA IgG in the group with leptospirosis.

Interference of *Escherichia coli* antibodies with *Borrelia burgdorferi* serology

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It has been suggested that *E coli* antibodies may cross react with *Borrelia burgdorferi* antigens and by this means interfere with borrelia serology. We measured the occurrence of *E coli* antibodies by enzyme immunoassay in 80 normal controls, eight patients with *E coli* septicaemia, and 47 patients with *B burgdorferi* infection. More than half of the normal controls (49/80), patients with *E coli* septicaemia (6/8), and patients with *B burgdorferi* infection (33/47) had IgG antibodies against *E coli* (OD value >0·400 in 1:100 dilution). Antibodies of IgM or IgA class against *E coli* were less frequently detected. There was no statistical difference in the occurrence of *E coli* antibodies of any immunoglobulin class between the three groups.

The cross reactivity of antibodies between *E coli* and *B burgdorferi* was studied by absorbing the serum samples with *E coli* antigen before determination of *E coli* and *B burgdorferi* antibodies by enzyme immunoassay. Nine serum samples from normal controls and three from patients with *E coli* sepsis contained antibodies against both *E coli* and *B burgdorferi*. Of these 12 serum samples, four became negative for *B burgdorferi* antibodies after *E coli* absorption. None of the borrelia patients became totally seronegative after absorption: in three cases IgM and in one case IgG *B burgdorferi* antibodies were removed by absorption.

It is concluded that (*a*) antibodies against *E coli*, particularly in the IgG class, are common among the normal population, in *E coli* sepsis, and borreliosis; (*b*) elimination of *E coli* antibodies by absorption may improve the specificity of *B burgdorferi* serology, but it might in some cases cause false negative results, if the cross reactive antibodies have been elicited by *B burgdorferi* infection.

Cross reaction or result of polyclonal stimulation? Patients with Lyme borreliosis give false positive reactivity in campylobacter serology

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We collected 30 serum samples (A) with high levels and 36 samples (B) with lower but positive levels of IgM or IgG class antibodies against *Borrelia burgdorferi (B.b.)* from clinically diagnosed patients with Lyme borreliosis. In group (B) the diagnosis was also proved by polymerase chain reaction or culture. Thirty control serum samples (C) were collected from healthy blood donors. We measured IgM, IgA, and IgG antibodies to *Campylobacter jejuni (C.j.)*, salmonella (S), yersinia (Y) (pooled antigen containing *Yersinia enterocolitica* IX and *Y pseudotuberculosis* IA and III), and *Bordetella pertussis (B.p.)*. Numbers in the table present percentage of positivity in at least one immunoglobulin class (>2SD above mean).

	n	*B.b.*	*C.j.*	*S*	*Y*	*B.p.*
A	30	100	50	33	23	7
B	36	100	17	19	14	6
A+B	66	100	32	26	18	6
C	30	10	3	13	3	20

A=strongly seropositive and clinical Lyme borreliosis; B=moderately seropositive, clinical, and Lyme borreliosis proved by polymerase chain reaction or culture; C=healthy blood donors. Positive limit OD >0·500 in 1:100 dilution.

It is concluded that (*a*) antibodies against campylobacter are commonly present in serum samples of patients with Lyme borreliosis and especially in the samples with high levels of *B burgdorferi* antibodies; (*b*) antibodies against salmonella and yersinia show this same tendency; (*c*) this phenomenon may be a result of oligoclonal or polyclonal activation caused by *B burgdorferi* infection.

Serological follow up after treatment of borrelia infection

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Objective—To study the duration of seropositivity after treatment of borrelia infection.

Methods—Consecutive serum samples from 30 patients with erythema migrans, 20 patients with acute or chronic borrelia arthritis, and 21 patients with acrodermatitis chronica atrophicans were analysed with a capture IgM enzyme linked immunosorbent assay (ELISA) and an indirect IgG ELISA, both using *Borrelia burgdorferi* flagella as antigen. All patients were treated with antibiotics. Follow up time varied from four months to five years.

Results—All patients were seropositive at onset of or within a month from antibiotic treatment.

Erythema migrans: 21/30 had positive IgM and 21/30 had positive IgG before treatment. 9/21, 4/21, 3/21, and 0/21 had still positive IgM 3, 6, 12, and 36 months after treatment. One patient had positive IgM after1½ years of follow up. 11/21, 9/21, and 6/21 had still positive IgG 6, 12, and 36 months after treatment. 2/21 patients had slightly raised IgG after nine years.

Borrelia arthritis: 5/20 had positive IgM and all had positive IgG before treatment. Two patients were negative for IgM when tested after nine months and one patient when tested after four years. One patient had a significant decline of IgM OD value after two months but had still positive IgM after 4½ years and the fifth patient had a significant decline in IgM after six months but had still positive IgM. In only one patient IgG declined to below cut off during follow up after four years. Significant decline of IgG OD values was found in 1/20, 7/20, 10/20, 12/20, and 14/20 patients after ½, 1, 1½, 2, and 4 years.

Acrodermatitis chronica atrophicans: 1/21 had positive IgM and all had positive IgG before treatment. IgM declined to below cut off after 1½ years. IgG OD values remained positive in all 21 patients. Significant decline of IgG OD values was seen in 2/21, 6/21, 9/21, and 10/21 after 1, 2, 3, and 4 years.

Conclusion—After treatment of late manifestations of borrelia infection, like acrodermatitis chronica atrophicans and chronic borrelia arthritis, serum IgG OD values remain positive for a long time. Second stage Lyme borreliosis, like acute borrelia arthritis, shows more rapid significant decline of serum IgG OD values, though seropositivity may remain for several years. A first stage manifestation, like erythema migrans, remains seropositive for a much shorter time with a few exceptions.

Comparative evaluation of three Lyme disease test kits

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Western blot test kits for Lyme disease (available for research only) from three companies were tested blindly with five CAP proficiency samples to evaluate their performance.

All three test kits use basically the same procedures: incubation with primary serum antibody at 1:100 dilution, followed by the conjugated secondary antibody, then development in substrate. All three companies use basically the same 31, 34, or 41 kilodalton bands for positive classification, with slight variations. After decoding, test kits from companies a and c agreed 5/5 for the presence of both IgG and IgM antibodies. The test kit from company c detected a two- to fourfold higher level of antibody in serum. The test kit from company b agreed 5/5 for the presence of IgG antibody, but failed to detect any IgM in the CAP positive serum samples (0/3). Test kits did not show cross reactivity when testing normal serum or serum from patients with antibody to rheumatoid factor or syphilis.

Our study shows that even though no false positive results were detected by any test kit, the possibility of false negative results exists with the test kit from company b.